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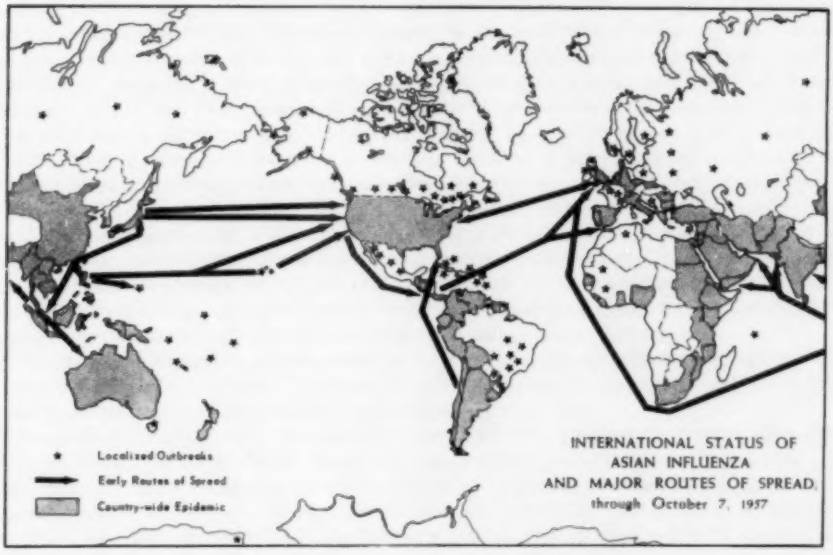
INTERNATIONAL CONFERENCE ON ASIAN INFLUENZA

USPHS

NATIONAL INSTITUTES OF HEALTH

Clinical Center Auditorium

Bethesda, Maryland · February 17, 18, 19, 1960



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FOREWORD

Through the years, man's life has been dramatically influenced by the sudden appearance of influenza in epidemic and pandemic forms. On each such occasion medical science has bestirred itself to study the disease intensively. Traditionally, however, within a short span of time thereafter the upsurge of interest in influenza research has begun to wane, leaving only a few scientists with special research interests to continue the elucidation of this particular disease. In spite of this feast-or-famine approach, much has been learned and large strides have been taken toward a more complete understanding of the disease process.

Because of this traditional pattern of influenza research, as well as other program considerations, the U. S. Public Health Service established an Advisory Committee on Influenza under the chairmanship of Dr. Colin MacLeod. During the deliberations of this Committee it became apparent that several actions should be taken to ensure a sustained program of influenza research and an effective effort at control. The first recommendation of the Committee was to hold an International Conference on Asian influenza bringing together many of the world's leading authorities on influenza research. These scientists were to report on their scientific observations made during the pandemic of Asian influenza and to point out neglected areas of research for special attention during the coming years. This recommendation became a reality and the proceedings of that meeting are herewith presented in their entirety.

Under the chairmanship of Dr. Clayton Loosli and the joint sponsorship of the National Institute of Allergy and Infectious Diseases and the University of Southern California School of Medicine, this Conference has resulted in a significant contribution to the knowledge of influenza, and it is believed that this report will represent a reference work for students and scientists of influenza for years to come. Within the pages of these proceedings dramatic evidence may be found of more precise epidemiologic, clinical, and basic research. Despite the inability to exploit fully the research opportunities of the pandemic, a significant body of new material has been added to our existing knowledge of influenza. Fully as important, however, is the clear evidence calling for special emphasis in certain poorly explored areas. Included are such important subjects as: (*a*) the problem of physiologic and biochemical responses to infection; (*b*) the influence of influenza on other diseases states, such as mitral stenosis; predisposing to difficulties during times of infection; (c) better information on the effect of virus in infected cells; (*d*) the basis of viral-bacterial enhancement; (*e*) studies of other possible hosts and reservoirs of the virus; (*f*) the role of inapparent infection and provocation; (*g*) the questions surrounding multiple antigens, routes of administration and schedules, and improved procedures for vaccine preparation; and (*h*) studies of chemical prophylaxis and therapy.

Although double jeopardy is not permitted under the law, it is permissible in medical research. Few will doubt that the International Conference on Asian Influenza has charted a course by which this disease is placed in danger of being both understood and controlled.

The second recommendation of the Committee was to develop a program of sustained research effort. Hopefully, this will be accomplished through the creation of a special group of influenza investigators whose purpose is to encourage the scientific community to pursue investigations in specific areas of influenza research where there exist gaps in our scientific knowledge. The direction of the program to be pursued by this Committee is charted largely within the pages of the Conference report. Not only did the International Conference represent an opportunity for the scientists to meet and exchange new research information and to create a definitive work of reference in this field, but it also contributed to the total research effort being mounted by the National Institutes of Health with the consultation of these special committees.

The National Advisory Allergy and Infectious Diseases Council and the National Institute of Allergy and Infectious Diseases are happy to have the privilege of participating in this total effort and to have been able to contribute staff, scientists, and funds to the Conference. It is with pride that we collaborate with the scientists of the world in this undertaking.

December 9, 1960

JUSTIN M. ANDREWS
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CONTENTS

FOREWORD ... 1
List of Participants ... v
Introduction. CLAYTON G. LOOSLI ... 1

GLOBAL EPIDEMIOLOGY

Epidemiology of Asian Influenza. With Special Emphasis on the United States. ALEXANDER D. LANGMUIR ... 2
General Discussion ... 10
Mortality from Influenza, 1957–1958 and 1959–1960. C. C. DAUER AND R. E. SERFLING ... 15
General Discussion ... 26

MECHANISMS OF SPREAD

The Mechanism of Spread of Asian Influenza. WILLIAM S. JORDAN, JR. ... 29
General Discussion ... 35
Animal Influenza. JAMES H. STEELE ... 41
Influenza in Swine and Horses. MORRIS SCHAEFFER AND ROSLYN Q. ROBINSON ... 47
Influenza of Horses. E. R. DOLL ... 48
General Discussion ... 50

CLINICAL CHARACTERISTICS

Twenty Years of Influenza Epidemics. C. H. STUART-HARRIS ... 54
General Discussion ... 61

PHYSIOLOGIC ABNORMALITIES

A Study of the Response of the Cardiovascular System to Asian Influenza. GEORGE E. BURCH, JOHN J. WALSH, AND WILLIAM MOGABGAB ... 68
General Discussion ... 77
Physiologic Abnormalities of Influenza. Lessons Learned and Future Needs. DAVID P. EARLE ... 79
General Discussion ... 81

PATHOLOGY

Broad Aspects of the Pathology and Pathogenesis of Human Influenza. J. F. PH. HERS AND J. MULDER ... 84
General Discussion ... 95

EVENING SESSION

Influenza in Perspective. THOMAS FRANCIS, JR. ... 98

STRAIN CHARACTERISTICS

Strain Characteristics of Asian Influenza Virus. ROSLYN Q. ROBINSON ... 105
General Discussion ... 109

METHODS OF DIAGNOSIS

Laboratory Diagnosis of Influenza by Virus Isolation. EDWIN H. LENNETTE ... 116
Diagnosis of Influenza by Serologic Methods. KEITH E. JENSEN ... 120
The Hemadsorption Technique, with Special Reference to the Problem of Naturally Occurring Simian Parainfluenza Virus. R. M. CHANOCK, H. M. JOHNSON, M. K. COOK, D. C. WONG, AND A. VARGOSKO ... 125
Diagnosis of Influenzal Infection by Means of Fluorescent Antibody Staining. CHIEN LIU ... 130
General Discussion ... 133

CRITIQUE OF DIAGNOSTIC SYSTEMS

Influenza. Diagnostic Procedures and Laboratory Organization, 1957. MORRIS SCHAEFFER ... 139
Diagnostic Laboratory Organization: Future. C. H. ANDREWES ... 142
General Discussion ... 143

SPECIFIC PROPHYLAXIS

Inactivated Influenza Virus Vaccines. Past, Present, and Future. FRED M. DAVENPORT ... 146
General Discussion ... 151

PROBLEMS

Influenza Virus Vaccine Production. I. WM. MCLEAN, JR. ... 157
Some Problems in the Standardization and Control of Influenza Vaccine in 1957. RODERICK MURRAY ... 160
Problems of Allocation and Distribution of Influenza Vaccines. DORLAND J. DAVIS ... 168
Public Acceptance of Influenza Vaccination Programs. IRWIN M. ROSENSTOCK ... 171
Asian Influenza Vaccination. Dosage, Routes, Schedules of Inoculation, and Reactions. GORDON N. MEIKLEJOHN ... 175
Some Results of the Study of Asian Influenza. V. M. ZHDANOV AND V. D. SOLOVIEV ... 178
General Discussion ... 187

ROLE OF HEALTH ORGANIZATIONS IN THE FUTURE

Influenza Pandemic. Preparedness Plans of the Public Health Service. L. E. BURNEY ... 193
The Role of Military Medical Services in Future Epidemic Influenza. JOSEPH H. MCNINCH AND R. P. MASON ... 198
Influenza. The Role of the State Health Officer in the Future. MALCOLM H. MERRILL ... 201
The Role of the Physician in an Epidemic of Influenza. JULIAN P. PRICE ... 204
The Role of Hospitals. EDWIN L. CROSBY ... 206
The Impact of Influenza on Industry in General. W. P. SHEPARD ... 209
Essential Industry and Its Role in an Influenza Epidemic. NORMAN PLUMMER ... 211
The Role of Communications. Television, Radio, and Others. ROBERT S. KLECKNER ... 215
General Discussion ... 216

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INTRODUCTION

DR. CLAYTON G. LOOSLI (Los Angeles): Ladies and gentlemen, on behalf of the Institute of Allergy and Infectious Diseases and the University of Southern California, may I welcome you to this International Conference on Asian Influenza. Dr. Norman Topping, President of the University of Southern California, and a former member of the National Institutes of Health, confers his approval in the following telegram received this morning. I quote: "I am happy that the University of Southern California can jointly sponsor this Conference on Asian Influenza. Please extend best wishes to the conferees and my regrets that I cannot be with you."

At this time, I would also like to thank the members of the Conference committee who worked so cooperatively together in developing the program. I refer to Drs. Fred Davenport, Alto Feller, Colin MacLeod, Joseph Smadel, and Charles Smith. The committee had the valuable services of others, including Drs. John Dingle and Thomas Francis, in preparing the agenda. Drs. Justin Andrews, Paul Petersen, and other staff of the Institute were most helpful in arranging the details for the Conference at this Clinical Center.

We are fortunate in having representatives from several foreign countries participate in this Conference. Each is an expert in some aspect of the general problem of epidemic influenza. These representatives include Drs. C. H. Andrewes and C. H. Stuart-Harris from England; Hideo Fukumi from Tokyo; Abraham Horowitz, Director of the Pan American Sanitary Bureau; J. Mulder from Holland; Fred Nagler from Canada; A.M.-M. Payne and M. M. Kaplan representing the World Health Organization, Geneva; and Professor V. D. Soloviev from Moscow. We are sorry that other of our foreign colleagues expert in the field of influenza could not be here. Those who are here, however, give this Conference worthy and truly international representation.

It is most appropriate that the Conference be international in character. Epidemic influenza, as exemplified by Asian influenza, recognizes no country boundary and spares no nationality or race of people. We know a great deal about the cause, the clinical and pathologic aspects, the prevention, and treatment of this disease. Yet the application of this knowledge is woefully inadequate.

Many of you do not appear on the printed program, but you have been invited to participate actively in the Conference. Feel free to discuss any or all aspects of it. We want the benefit of your experience and wisdom in an effort to formulate more effective means of control of epidemic influenza, which periodically contributes so heavily to the loss of lives of the peoples of this earth.

GLOBAL EPIDEMIOLOGY

CLAYTON G. LOOSLI, *Chairman*

EPIDEMIOLOGY OF ASIAN INFLUENZA[1]

With Special Emphasis on the United States

ALEXANDER D. LANGMUIR

Introduction

In planning for the presentation of the broad subject of the epidemiology of Asian influenza at this Conference, Dr. C. C. Dauer and Dr. R. E. Serfling joined with the present writer in preparing series of annotated tables and figures that serve to provide a description of many of the essential epidemiologic characteristics of the pandemic of Asian influenza in 1957–1958. These have been published in a brochure entitled "The Epidemiology of Asian Influenza 1957–1960" (1). Certain of the figures from this descriptive brochure will be reproduced in this report and in the paper by Drs. Carl Dauer and Robert Serfling, which follows.

The present discussion will be limited to four main themes which have been chosen to help organize a great mass of data into certain centralizing concepts with the objective of stimulating discussion and clarifying issues. These four themes are:

1. The remarkable prediction of May, 1957
2. The ability to trace the spread of the pandemic
3. The age distribution of cases
4. The unique second wave of mortality

The Remarkable Prediction

The Asian influenza pandemic apparently had its origin in Central China but became known to the rest of the world in April, 1957, when epidemic influenza occurred in Hong Kong. From there it rapidly spread to Singapore, Manila, Taiwan, and other areas of Southeast Asia. While being rather sharp and intense, these epidemics in themselves evoked no remarkable or unique comment until serologic study of the causative strains of virus was reported. Concurrently and independently, Dr. M. R. Hilleman in Washington, Dr. C. H. Andrewes in London, and Sir Macfarlane Burnet in Melbourne (2) reported that the strains were related to type A but were serologically quite distinct from the A1 strains that up to that time had been prevalent throughout the world for the past decade.

The significance of this finding was immediately appreciated by all epidemiologists and virologists interested in influenza. The long anticipated introduction of a new pandemic strain had occurred, and it had been recognized in time to trace its spread and even to plan control measures. There was essentially unanimous agreement with the predictions that epidemics would continue to spread through Southeast Asia and subsequently encircle the Southern Hemisphere in the then winter months. It was further recognized that there would be repeated "seeding" of the virus into the countries of the Northern Hemisphere and that localized epidemics would be recognized, but that extensive community-wide epidemics would be deferred until the fall.

To some, these predictions seemed simple, elementary, and even obvious; but from a more detached scientific view it is difficult to match in the history of epidemics a more comprehensive and all-embracing prediction. The extent to which the prediction was fulfilled by the subsequent course of events testifies to the extent of modern knowledge and understanding of influenza.

Tracing the Spread

The spread of the pandemic throughout the world is shown vividly in the map (figure 1) which is based on the map prepared and presented by Dr. A. M.-M. Payne (2), who will elaborate on the spread of the pandemic in countries other than the United States. Epidemics spread directly and rapidly from Hong Kong to contiguous countries in Southeast Asia. Spread by boat from seaport to seaport was apparently more effective than by airplane. Seeding of the virus into Europe and the United States occurred in May and June, and many

[1] From the Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Atlanta 22, Georgia.

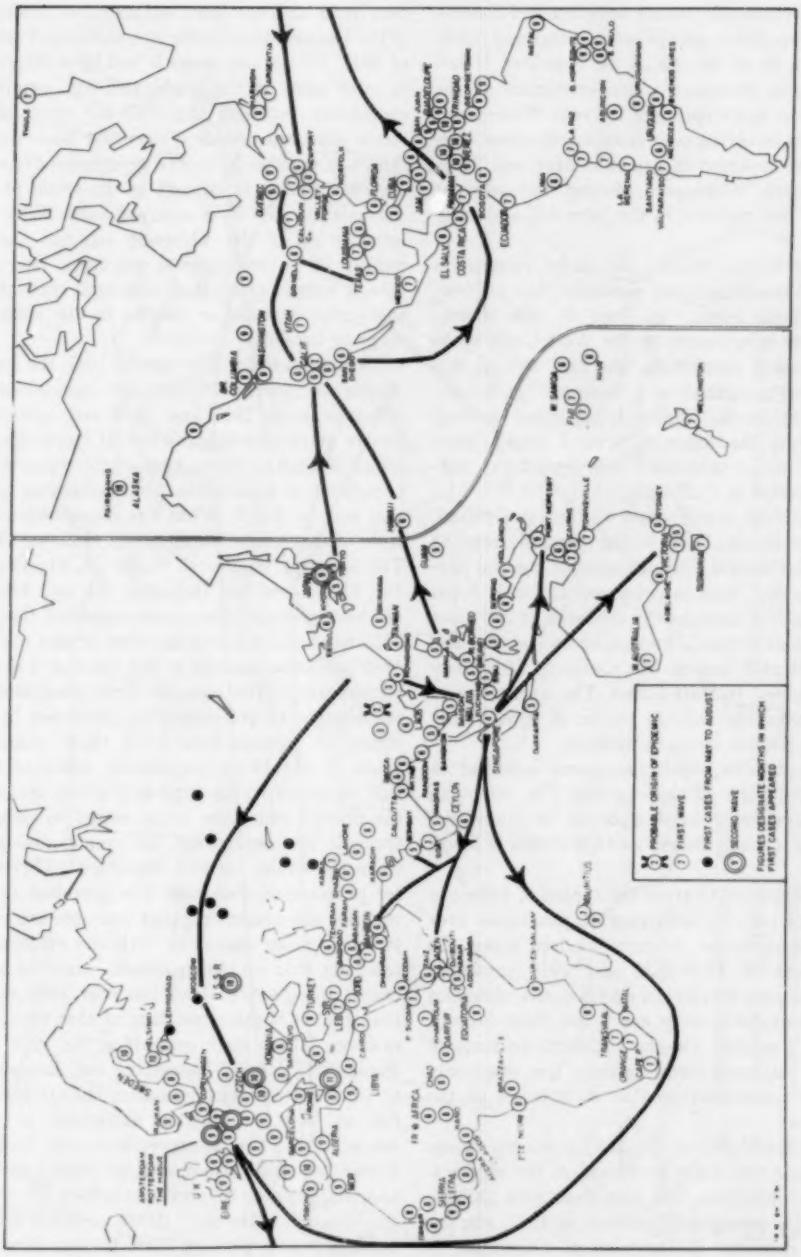
PROBABLE ORIGIN OF EPIDEMIC
FIRST WAVE
FIRST CASES FROM MAY TO AUGUST
SECOND WAVE
FIGURES DESIGNATE MONTHS IN WHICH FIRST CASES APPEARED

FIG. 1. Progress of Asian influenza pandemic, February, 1957 to January, 1958.

localized outbreaks among crowded and concentrated population groups were recognized. Only in Japan, of all nations in the Northern Hemisphere, did community-wide epidemics of the virus occur in the spring of the year. Widespread epidemics involving large numbers of school children were reported in Japan in May and June. These were interrupted during the summer months, but recurred in the later fall and early winter.

In the United States, the initial recognition occurred among military personnel, first at Newport, Rhode Island, on June 2; but shortly thereafter it appeared on the West Coast without traceable connection. The first civilian outbreak was recognized at a "teen-age" girls' conference at Davis, California, beginning on June 20. During the following several weeks, more than 15 similar outbreaks were reported in childrens' camps in California.

In late June, a conference was held in Grinnell, Iowa, involving 1,800 young persons from 43 states and several foreign countries. Several participants had been in prior contact with Asian influenza in California. An outbreak of 200 cases occurred at Grinnell, and an additional 50 cases were detected among the conferees after they had returned to their homes. The occurrence of early outbreaks and the routes of spread that were traced are shown in figure 2.

In September, epidemic spread occurred in almost all parts of the country. The extent to which epidemics were reported by individual counties through December 16 is shown in figure 3.

This capacity to trace the spread of influenza is one of the characteristics of pandemics over the past centuries. In more modern times, the pandemics of 1889–1891 and 1918 presented these features vividly. In contrast, the epidemics of influenza that recur every few years are not readily traceable. On the contrary, evidence of widely scattered virus activity has commonly been demonstrated months in advance of the epidemic.

The traceability of the 1957 pandemic brings into focus two basic problems in the epidemiology of influenza. The first deals with the absence of a recognized pandemic in 1947, and the second concerns the problem of identifying in retrospect the specific strains of virus that cause pandemics.

The A1 strain of influenza virus produced the very mild and scattered epidemics of influenza in the United States in the late winter and spring of 1947. Within two years it had been identified in most parts of the world and had seemingly completely replaced the PR8-WS type of A strain that had prevailed since the discovery of influenza virus in 1934. The geographic origin of the A1 strain is as obscure as the origin of the Asian strain in China seems definite. Why the introduction of the A1 strain was not accompanied by a recognizable pandemic and yet spread widely, or at least appeared throughout the world, remains an enigma in the epidemiology of influenza.

Notwithstanding the events of 1947, the recognizable occurrence of traceable pandemics of influenza as in 1957 and 1918 and 1889–1891 clearly marks the introduction of a new specific strain of virus to which a high proportion of the population is susceptible. The challenging question may be asked: What was the specific character of the strains introduced in 1889 and 1918? The serologic studies of Shope (3, 4), Francis (5), Davenport (6), Hennessy (7), and Mulder (8) have provided some clues suggesting that the 1918 pandemic was due to swine strains and the 1889 pandemic was due to the Asian or a closely related strain. The basis for these conclusions is the absence of strain-specific antibodies in the serum of persons born after these pandemic years. It should be emphasized, however, that this retrospective-age-antibody study measures the time at which the strain ceased to circulate through the population. It cannot determine when the strain was first introduced. Therefore, for purposes of discussion it is pertinent to ask whether the present popular concepts are valid. It seems more consistent with the evidence to conclude that an Asian-related strain of virus ceased to circulate about the year 1890 rather than that it began circulating at that time. The evidence is less clear concerning the 1918 pandemic. The data of Davenport and Francis (5, 6) show that persons born after the early 1920's fail to have swine-strain antibodies in their serum. Thus, two interpretations are possible. Either the swine strain was introduced in 1918 and continued to be prevalent only a few years, disappearing in the early 1920's, or it was present and circulating prior to 1918. Present data permit no conclusion as to when it was introduced but only the approximate time of its disappearance. Similarly, it is possible to conclude that the

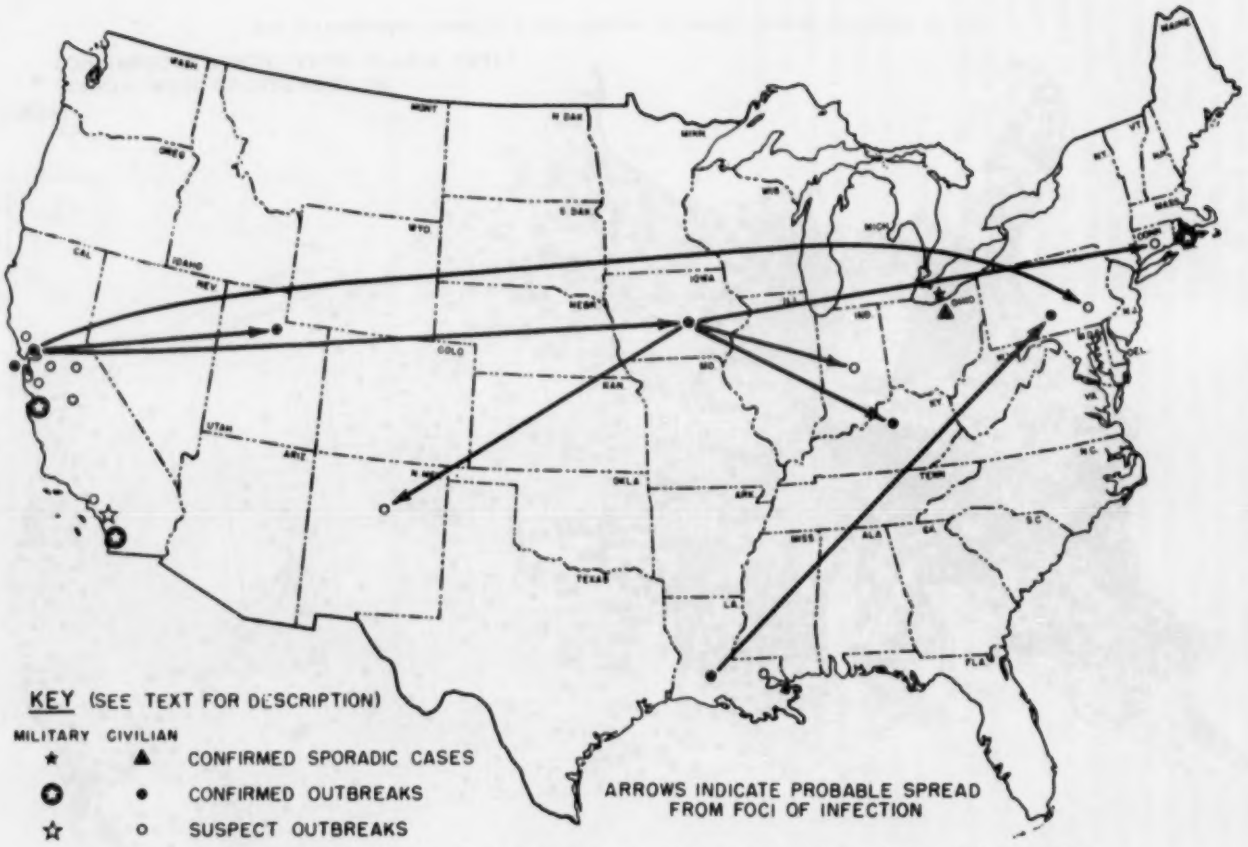

FIG. 2. Status of Asian influenza in the United States and major routes of spread, through July 22, 1957.

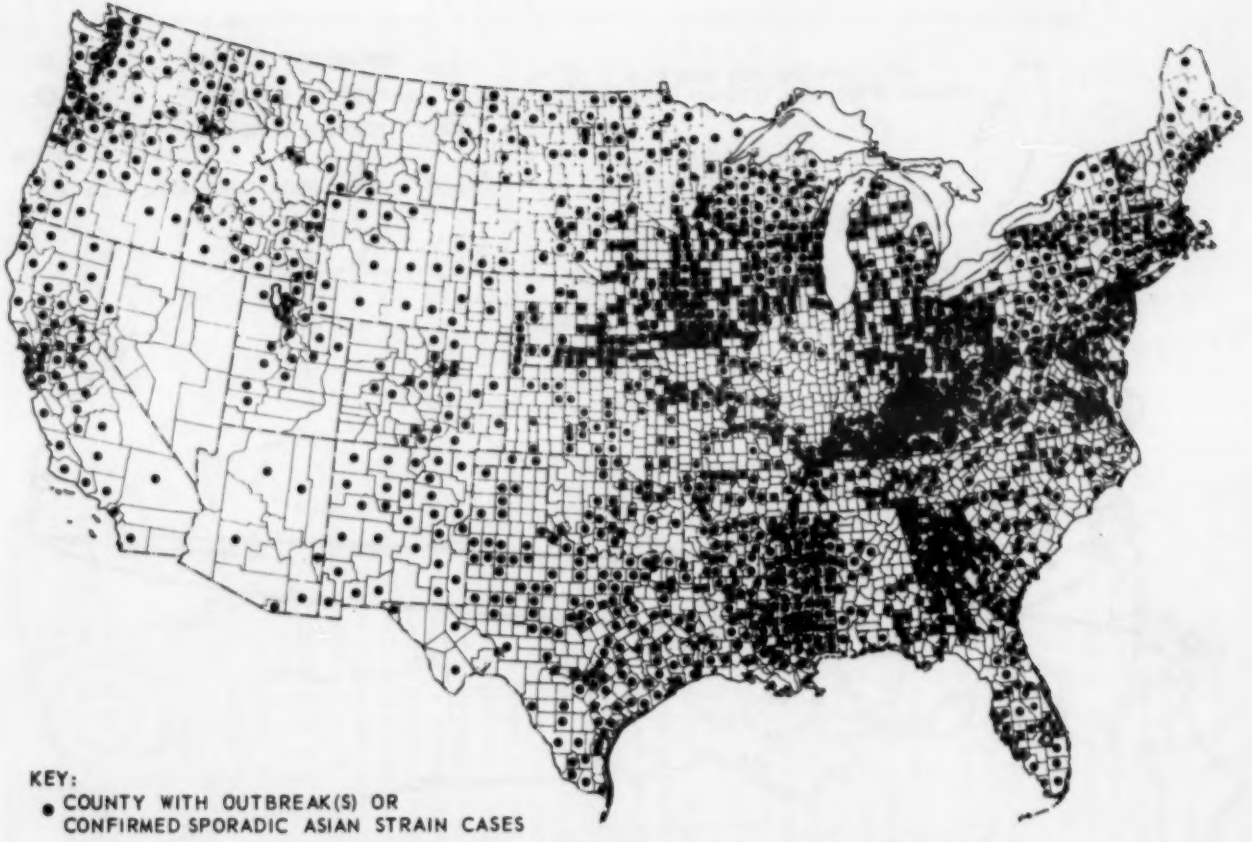

FIG. 3. Geographic spread of Asian influenza by county through December 16, 1957.

WS-PR8 strain disappeared after the 1943 epidemic. It was clearly prevalent in 1933 and 1934, but when it was first introduced is only conjecture. One may properly ask: Could this strain have been the cause of the 1918 pandemic and continued its world-wide prevalence for twenty-five years? There was a remarkably consistent epidemiologic pattern of periodicity during this quarter of a century (9). It is also possible, of course, that "silent" nonpandemic-producing strains comparable to the A1 strain of 1947 might have been introduced at unrecognized times between the pandemics.

Age Distribution

Unusual opportunity was presented during the Asian influenza pandemic to measure the age distribution of clinical cases. Community-wide epidemics occurred in Tangipahoa Parish, Louisiana (10), in August, and numerous epidemics were recognized in many parts of the country in September. These early epidemics permitted the identification of cases of clinical influenza when other febrile nonspecific respiratory diseases were infrequent. Thus, the simple and classic type of epidemiologic survey had special value. Small-scale surveys were performed throughout schools in Louisiana and in Kansas City (11) (figures 4 and 5). The National Health Survey also collected data for a random sample of the national population (figure 6). A characteristic pattern was observed.

In Tangipahoa Parish, Louisiana, and in Kansas City, the age-specific attack rates for influenza were low among preschool children, rose markedly among primary and secondary grade school children, and reached a peak among high school students and young adults. The rates then fell progressively with increasing ages in adult life. In the National Health Survey, the epidemic peak in October and early November struck most heavily among those less than fifteen years

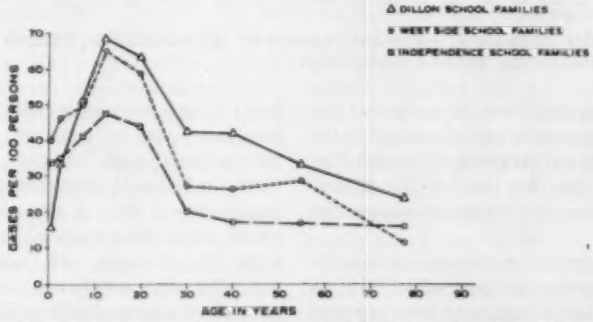

Fig. 4. Influenza attack rates by age: Tangipahoa Parish, Louisiana, August, 1957.

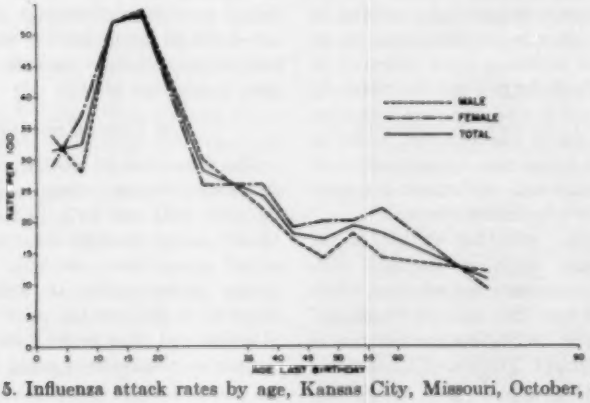

Fig. 5. Influenza attack rates by age, Kansas City, Missouri, October, 1957.

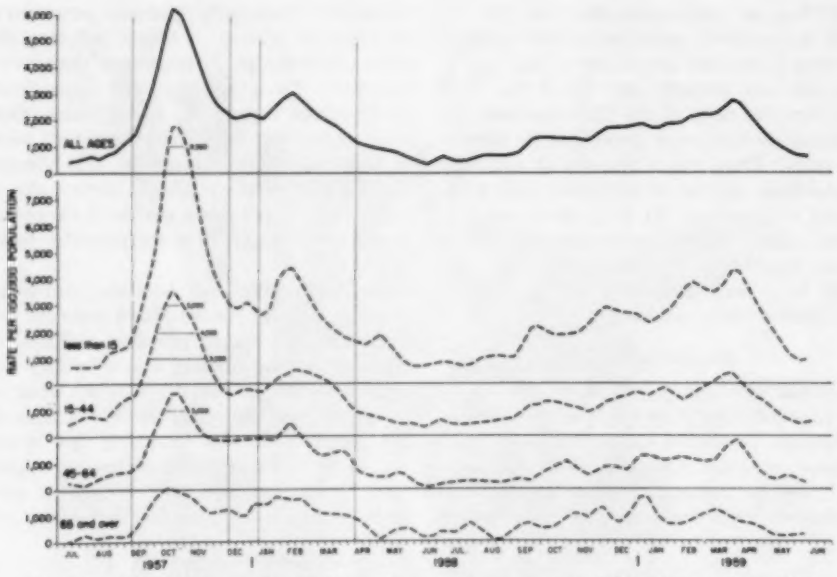

FIG. 6. Weekly incidence of respiratory illnesses by age groups, July, 1957, to June, 1959. (Data provided by the National Health Survey)

of age. Among adults fifteen to forty-four the epidemic was considerably less severe, and in the older age groups it was progressively milder. Unfortunately, the data for the less-than-fifteen-year age group were not fractionated into smaller age groupings.

The progressively falling incidence rates of influenza with increasing age have often been ascribed to maturation of immunity from repeated past experience with related strains of virus. This conclusion is based upon an unproved assumption that exposure is essentially uniform at all ages because virus is so widespread in an epidemic. The low incidence rates observed in 1957 among preschool children and the relatively low rates among the five-to-nine-year-olds compared with those ten to nineteen cast doubt on the validity of the maturation-of-immunity concept. It is clear that wide variations in opportunity for exposure to infection occurred.

In the increasingly suburban culture of the United States today, there are obviously wide variations in the opportunity for infection, which are a function of age. The modern "teenager" probably experiences the highest contact rate of any population segment. The consolidated school system, especially high schools, plus school buses and the high intrinsic mobility of the average American youth is "designed" to spread a contagious infection like influenza. The number and variety of different close contacts each day must greatly exceed that of the housewife, her preschool child, and even her husband in his daily work. The closeness and "intimacy" of school and school-bus contacts probably now exceed even those among members of the same household. The benefits of the popular concept of "family togetherness" may even extend to a low risk of acquiring influenza in the home. Crowding—perhaps rather intense crowding—was apparently necessary to the spread of Asian influenza in epidemic form.

THE UNIQUE SECOND WAVE

The recurrence of second and third epidemic waves of influenza became popular concepts in the 1889–1891 and 1918–1920 pandemics (3, 4, 12, 13). In the virologic era since 1933, however, second waves have not been described as intrinsic characteristics of influenza epidemics. Many have doubted the validity of the concept of second and third waves, believing instead that the earlier observations could best be explained on the grounds of the sequential occurrence of

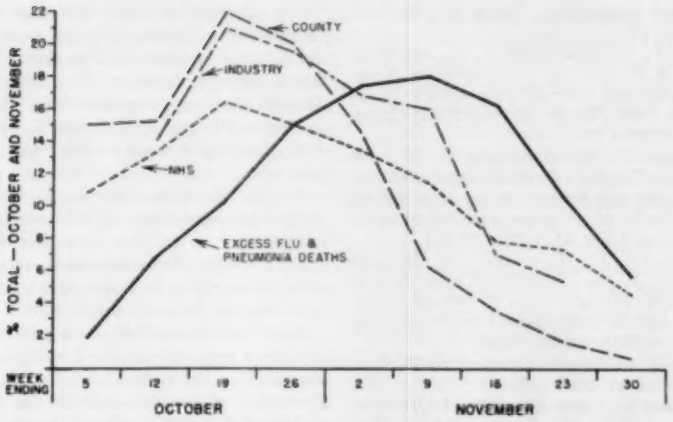

FIG. 7. Morbidity and mortality peaks, Asian influenza, United States, 1957.

epidemics due to specifically different viruses. The experience in 1957–1958, however, established a second wave as an important and serious feature of this pandemic.

In the United States the first epidemic wave began in September. The several indices of morbidity, county reports, industrial absenteeism, and the National Health Survey, all showed peaks in October (figure 7). The traditional index of epidemic influenza, namely excess mortality, was at its peak in November. In December all indices of influenza declined. Early in 1958, however, a marked wave of excess mortality occurred in all parts of the country except the mountain and Pacific states. This second wave, as measured by excess death rates, was almost as severe as the first wave, but it was not accompanied by other indices of epidemic influenza. School and industrial absentee rates were normal for winter months. Epidemics and outbreaks of febrile respiratory disease were not being reported with undue frequency. The data by age group from the National Health Survey, that became available later (*see* figure 6), reveal rates that are essentially similar in their level to those observed during the following winter months of 1958–1959.

At the time, the causes for this unprecedented wave of mortality were obscure, but on retrospective study it seems clear that Asian strain influenza virus was circulating widely among older adults and elderly persons (14, 15, 16) who were apparently quite susceptible to infection and to disease. In this age group the risk of fatal complication is markedly higher than among the high school children and young adults who were selected for attack in the first wave.

The mechanism of spread in the second wave and the factors maintaining a wide dissemination of virus over a period of months and throughout a large part of the nation are far from being clearly explained. One may ask: Is the second wave of mortality a characteristic of the Asian strain, distinct from the A and A1 strains of recent experience? Is the second wave a manifestation of pandemic influenza that occurs in the late summer and fall months and involves primarily school children but not adults?

From the accuracy of the global predictions made in the spring of 1957, we may conclude that there is a substantial body of scientific knowledge about epidemic influenza, but we may also look into the future with an awareness that there is much still to learn before this great public health problem is brought under effective control.

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General Discussion

Dr. Hideo Fukumi (Tokyo, Japan): It is not precisely known when Asian influenza virus was introduced into Japan for the first time. Following its introduction there were two epidemic waves. Why? The first outbreak of Asian influenza recognized as such in Japan started on May 10, 1957, in an elementary school in Tokyo, and then the whole country became rapidly involved in the first epidemic wave, which lasted until July. The second wave began in September and apparently subsided in December, but a small recurrence was recognized in early 1958. The data are summarized in a report published in the *Bulletin of the World Health Organization* (1959, *20*, 187). To recapitulate briefly, during the fall of 1956 and the early part of 1957 the influenza outbreaks were due to influenza viruses A1 and B. The Asian influenza epidemic began in Japan in May, 1957, and extended through the early months of 1958. There were two epidemic waves of approximately the same magnitude, the second being a little larger than the first one.

It may be said that the second wave chose only those areas where no influenza cases had been encountered in the first wave, but this holds true only partially. For example, a large number of schools, especially in large cities, were found to be attacked by both epidemic waves, but in these cases it can be said that those individuals who had not been attacked by the first wave were mostly involved in the second one, though there were a few cases of reinfection. Such was true not only in schools but also in other places such as factories, offices, et cetera.

Serologic examinations revealed in general that about 20 per cent (4.6 to 38.2 per cent) of the workers in health centers had antibody against A2 virus after the first epidemic wave, and about 45 per cent (25.7 to 61.8 per cent) after the second wave. It was also shown that 50.3 per cent of the persons in camps of Japanese Self-Defense Forces had A2 antibody after the first wave, while 72.3 per cent had A2 antibody after the second wave. These findings are considered to indicate that, in workers in health centers, the invasion of the A2 virus progressed in the first wave until about 20 per cent of the population became refractory to the infection, while it proceeded further until 45 per cent of the population were refractory to it in the second wave. In general, then, the progress of the epidemic was stopped when the number of those rendered refractory to the infection due to recovery from it reached a certain level, thus protecting the remaining population from infection. This level varies, depending upon the nature of transmission-modus, environmental conditions, et cetera. It may thus be concluded that the value of this level was about 20 and 45 per cent for the first and second waves, respectively, for workers in health centers; whereas it was 50.3 per cent and 72.3 per cent, respectively, for the two waves for members of the Self-Defense Forces camps.

The reason why the value of the level differs for the first and second waves, respectively, is presumed to be as follows: in the first epidemic wave, droplet infection (that is person-to-person infection) was the main modus of transmission due to the warm weather; whereas, in the second wave, droplet *nuclei* infection (that is infection due to air contamination) played an important role together with droplet infection because of rather close living conditions in the cold weather.

Generally speaking, there was reported to be a phase of virus seeding after virus introduction in European and American countries, and in such countries the main epidemic period started with cold weather in late September or early October. In Japan the populations were not so crowded and droplet infection alone appeared to cause widespread outbreaks and was responsible for the fairly heavy first epidemic wave immediately after virus introduction, with no lag phase of virus seeding before the outbreak.

Dr. Abraham Horowitz (Washington, D. C.): As Dr. Langmuir has very well pointed out, at the end of March, 1957, it was clearly predictable that influenza epidemics would encircle the Southern Hemisphere during the winter months, i.e., before October of the same year. The national influenza centers were alerted and the interest of the national public health services of the southernmost countries of the American continent was further aroused by the visit of the Influenza Study Group of the U.S. Armed Forces Epidemiological Board. A few careful epidemiologic studies were made in those countries, and a summary description will be given later of the observations made in Chile by the Chilean Committee on Influenza in cooperation with the U.S. Influenza Study Group and the School of Medicine of the University of Concepción, and with the support of the Pan American Health Organization.

In general, however, the control programs in the countries of Middle and South America were not as comprehensive and effective as they were in Canada and the United States. This was partly due to the earlier start of the epidemics and partly to the scarcity of appropriate technical facilities for the production of influenza vaccine and to the limited resources of most of the public health services. Small amounts of influenza vaccine were produced and administered to selected groups of people in Chile, Argentina, and Brazil.

A2 influenza virus was isolated at the national influenza centers of several countries and at a few other laboratories, as shown in the attached map (figure 8).

The figures in the map indicate the week of the year when the incidence of influenza began to increase. The disease appears to have started its spread in the Continent from two different areas: (*a*) somewhere around the border of Bolivia, Chile, and Peru (spotted area in the map); La Paz was the first important city attacked during the second week of July; (*b*) Mexico, where the starting areas and dates have not been well defined.

From the Bolivia-Chile-Peru border the disease spread in three directions:

(*1*) Toward the interior of Bolivia.

(*2*) Toward the west and north of Peru and then to the northeast of the same country. Incidence, as determined by stratified surveys, was highest in Arequipa (72 per cent); in Lima it was 54 per cent. Excess mortality was evident only in the areas of Puno and Junin, both having cities above 10,000 feet.

(*3*) Toward the south of Chile and then to the neighboring cities: Antofagasta, Santiago, Valparaíso, Concepción, and Magallanes were invaded in succession.

An epidemiologic survey in 1 per cent of the population of Santiago showed an incidence of 65.5 per cent in 15,000 people with higher attack rates in school-age children. Infants and elderly people were less affected (45–50 per cent).

A twofold increase in deaths took place during the epidemic and most of that increase was attributable to influenza and pneumonia. From July 15 to September 1, there were in Santiago 1,430 influenza and pneumonia deaths as compared with 281 in the same period of the previous year. The elderly contributed predominantly to the fatalities, but there was an increase in deaths in all age groups.

In the rest of the country the picture was very similar. In spite of all this, the death rate for the year 1957 (13.7 per thousand) was only slightly higher than the rates for 1956 (12.8 per thousand) and 1958 (13.2 per thousand).

Although a variety of organisms was associated with the fatal cases of pneumonia, *Staphylococcus aureus* was the one most frequently isolated. A small proportion of fatalities was probably the result of primary influenzal pneumonia. Serologic studies showed that the majority of the patients with pneumonia developed antibodies against A2 influenza virus.

The A2 strain isolated in the United States arrived in Chile when the epidemic was well under way and, therefore, influenza vaccine was not readily available for administration to the general population. Had it been so, however, a control program of this kind would have been quite expensive, and one certainly wonders whether a country with so many important health problems and economic limitations should have given a high priority to influenza vaccination in preference to other more permanent and effective health measures.

The first city affected in Argentina was Mendoza, across the Andes mountains, where the incidence was very high and the severity of the disease did not differ much from the Chilean epidemic.

From there on, and in rapid succession, the epidemic spread to the North (Salta and Tucumán) and to the West (Córdoba and Buenos Aires). Spread to the rest of the country took place later. In general the incidence was not very

INCREASED INCIDENCE OF INFLUENZA STARTED AT THE 32nd. WEEK OF THE YEAR 1957
INFLUENZA A2 VIRUS ISOLATED
SMALL ISOLATED OUTBREAKS

FIG. 8. Progression of Asian influenza in Middle and South America.

high in most of Argentina (estimated as below 35 per cent in most cities), and the disease was mild.

The Uruguayan cities on the shore of the River Plate were affected soon after Buenos Aires and acted as centers of spread to other localities in the interior of Uruguay.

Outbreaks in Paraguay took place later and they apparently were related to the routes of communication and traffic between that country and Argentina.

The first outbreaks in Brazil took place in the South: Uruguayana (near the Argentine border) and Pôrto Alegre. Two weeks later São Paulo and Rio de Janeiro were affected, and the epidemic wave spread later on to other parts of the country.

This sequence, which might appear to be so well defined, could not be seen so clearly in other countries. This may be due to the fact that the whole southern area was in mid-winter and they therefore experienced "full size epidemics" early enough to allow a clear-cut routing of the spread.

According to the information received, El Salvador was the first country attacked in Central America just one week after Mexico, beginning on the Pacific coast. The disease spread afterwards to Guatemala and Honduras.

The southeast of the isthmus was affected later; the epidemic there appears to have started from the Pacific coast of Panama.

There have been no reports of influenza in Nicaragua during 1957.

Both Venezuela and Ecuador suffered two waves of upper respiratory infection outbreaks. There is not enough laboratory information available, so that we are unable to say if any of these waves was etiologically related to the A2 influenza pandemic. The timing and absence of spread of the early waves, which affected only some rural areas, suggests that they were not related to the pandemic.

Colombia was probably invaded from Panama. The Guianas and the Caribbean islands were affected in later outbreaks. It is very difficult to issue any hypothesis on the origin of these late spreads.

Dr. V. D. Soloviev (Moscow, U.S.S.R.): The 1957 pandemic of influenza broke out in some countries in the form of one wave and in other countries in the form of two waves. In the Soviet Union, the first wave occurred in the spring-summer season and the second wave in the autumn.[1] The first serious outbreaks of diseases were observed in the cities of the Central Asian republics; the city of Tashkent had the most intensive outbreak in May. The incidence of the disease increased nine-fold in May, as compared with the preceding months. In course of time, however, this type of Asian influenza was specific only to certain southeastern parts of our country. There was a comparatively small increase in the incidence of the disease in the rest of each territory. During the summer months, however, each outbreak left a widespread area of infection.

The second wave of the pandemic developed in late September and in October (particularly), when there was a maximal increase in the incidence of the disease. This outbreak was observed almost simultaneously in the majority of cities. At the same time, a notable, but considerably smaller increase in the incidence of the disease was observed in Tashkent. The same picture was also noted in other regions where a first wave of the pandemic was most intensive. The incidence of influenza was gradually increased in November and later in the year, but throughout the year it was higher than the normal level of previous years. After the lapse of thirteen or fourteen months, there was a general increase in the incidence of the disease in January and February, 1959, which quantitatively was lower. On the other hand, it was higher in some cities than during the pandemic of 1957. Judging by the principal factors, the outbreak of 1959 was not essentially different from pandemic influenza. School children and infants were taken ill in most cases. In the majority of cases, the clinical course of the disease was either mild or moderate. The highest mortality was not among small children and old people in 1959.

Laboratory investigations demonstrated that the strains of virus isolated were A2, corresponding in their antigenic structure to the strain of virus A2 isolated in 1957. However, one circumstance deserves special consideration. Among the cases of disease in 1959, there were people who had influenza in 1957. This group comprised 20 to 35 per cent in Moscow and up to 51.4 per cent in Kiev.

In summary, during the past three years Tashkent was the first city in which pandemic influenza occurred. The first outbreak happened in May 1957, and then to a rather lesser degree in the autumn of 1957, and then again in 1959. In Moscow the first outbreak occurred during the summer season, but the outbreak of highest incidence occurred in the autumn of 1957, and then again there was a very intensive outbreak in 1959.

Dr. A. M.-M. Payne (New Haven, Connecticut): I would like to refer to figure 1 of Dr. Langmuir's article. This map has been reproduced several times.[2, 3] It shows by the number enclosed in

[1] Zhdanov, V. M.: The 1957 influenza pandemic in the U.S.S.R., Bull WHO, 1959, *20*, 489.

[2] Payne, A.M.-M.: Some aspects of the epidemiology of the 1957 influenza pandemic, Proc Roy Soc Med, 1958, *51*, 1009.

[3] Payne, A.M.-M.: Global epidemiology today, Yale J Biol Med, 1959, *32*, 4.

each circle the month during which the first cases of an epidemic occurred as reported to WHO. The arrows show the presumed route of spread, emphasizing the importance of shipping. I should perhaps mention that the information contained in the map has an unequal value and reflects to some extent the care with which outbreaks, especially localized ones, were sought. An area without a circle means lack of information at the time the map was prepared and not that no epidemic occurred.

In the few minutes allotted, I can only outline some of the conclusions which I believe can be drawn from the mass of data obtained during and after the pandemic. To indicate the extent of these data, I would mention that my colleagues and I at WHO are preparing a bibliography on the Asian pandemic, and we have already collected more than 2,000 references.

My first conclusion is that we need to improve our international early warning system. The pandemic began in an area to which we had no access and is shown by the dark spot marked X in the southern part of China. In spite of the vigilance and the fine work of Hilleman in the United States, Hale in Singapore, the World Influenza Center in London, and the Walter and Eliza Hall Institute in Melbourne, we now know that we were nearly two months late in learning that a new virus was spreading. The Chinese virologists had already recognized its main features by the time the pandemic broke out in Hong Kong in mid-April. There are other areas in the world today to which we have access but where a lack of interest or facilities makes an early warning impossible at present. This at least should be recitfied.

Although the prediction of the world-wide spread of the virus was correct, I, for one, was wrong in assuming that initially the wave would sweep south where winter was beginning. Actually the spread was most rapid through the tropics, reaching the Middle East by June; whereas population-wide outbreaks did not start in Australia and South Africa until late July and in South America until July and August. It was striking that this rapid spread through the tropics was associated with outbreaks on ships and the massive introduction of the infection into the great ports of India, the Persian Gulf, and the Red Sea, whence it spread to other cities and later to rural areas. In these regions and elsewhere primary outbreaks around airports were unusual.

In the tropics, the first stage of the virus invasion was an epidemic phase in the general population which, I postulate, was the result of massive introductions into crowded communities. In contrast to this, in the United States and in Europe and probably—although less obviously—in Australia, the first stage was one of virus seeding, introduction occurring on a more limited scale, probably from the tail-end of outbreaks on ships and from aircraft. Outbreaks did occur during this stage in close aggregations such as camps; for example, the outbreaks mentioned by Dr. Langmuir and those in the Ambonese camps so well studied by Dr. Mulder in the Netherlands. At least one such outbreak occurred in Australia more than a month before the main epidemic there. These outbreaks did not immediately spread to the general population but, if the aggregations dispersed (as in the case of scout camps and youth festivals), they contributed to the further seeding of other areas. As pointed out by Dr. Langmuir, in many areas the opening of schools, that is to say, the creation of new aggregations already seeded with virus, signalled the start of the main wave.

In some countries of which we've heard—Japan and parts of the Soviet Union—there were two clear epidemic waves in which the areas most affected during the first wave were relatively spared in the second. In other countries, however, for example in the United States and in the United Kingdom, the main epidemic phase was followed after a few weeks' quiescence by what I would like to call an endemic phase, characterized by few recognizable outbreaks. Sporadic cases occurred which were associated with continuing activity of the virus as shown by repeated isolations for several months, and there was an excess of deaths attributed to influenza and pneumonia, predominantly in elderly persons who had esceped infection in the epidemic wave. This may have been due to their small contact with children and young adults who were specially involved in the epidemic phase. The delay in the time of their infection until the usual respiratory season in late winter may have increased the risk of pulmonary complications.

Finally, I will briefly mention quarantine which was attempted in a few countries, usually without any effect whatever. In South Africa, however, the infection did not apparently enter through the ports where the ships were quarantined, that is to say, the passengers and crew were not allowed to land. But it appeared to be brought down from the north, probably by the immigrant labor force. Some delay in the introduction of the epidemic was claimed. Israel presented an interesting situation. Politically isolated from her neighbors, the epidemic started in September almost two months after theirs and there seems to be no explanation for this other than the effect of isolation. Thus the entry of the infection may be delayed under certain circumstances involving severe disruption of international communication but it certainly cannot be prevented altogether.

MORTALITY FROM INFLUENZA[1]

1957–1958 and 1959–1960

C. C. DAUER AND R. E. SERFLING

INTRODUCTION

Morbidity data that can be obtained currently from numbers of cases reported, absenteeism records in schools and industries, or from sample surveys made in various ways, are useful as a rough index of the prevalence of influenza, but do not provide quantitative information through which the national and regional severity of an influenza epidemic can be measured. Mortality data, since they are collected systematically in epidemic and endemic periods, provide the only base for numerical estimate of the relative intensity of influenza epidemics. Excess mortality provides a valuable tool for study of epidemiologic characteristics of influenza epidemics and is also useful for current surveillance despite a lag from the peak of morbidity to the peak of mortality.

INFLUENZA AND PNEUMONIA MORTALITY IN LARGE CITIES

For a number of years a large group of cities in all parts of the United States have been reporting weekly to the Public Health Service the number of deaths from all causes and also from influenza and pneumonia. These reports indicated a rise in mortality from influenza and pneumonia in about 100 cities in the fall of 1957, which became apparent about four weeks after the rise in incidence of acute respiratory illnesses as determined by weekly sample surveys of the U. S. National Health Survey (figure 9). The rise in mortality in this early phase of the nation-wide epidemic began early in October, reached a peak in the first week of November, and then declined over the next six weeks, although not to the usual level. A second rise in mortality began toward the end of December, and the numbers of deaths reported increased more slowly, reaching a peak at the end of February, 1958, which was nearly as high as in the preceding November. A rise in incidence of acute respiratory illness occurred in mid-January but reached a maximum early in February, about three weeks before the peak of the second wave of mortality. In this phase the weekly estimates of respiratory illnesses did not approach the level reached a few months earlier.

The course of the epidemic is shown in figure 2, as measured by weekly reported deaths in 108 cities for the United States and nine geographic divisions. In this figure "expected deaths" are shown as a smooth solid line with an upper dotted line, the "epidemic threshold" which provides a guide to the upper limit of endemic variation. The procedure for constructing these curves has been described in detail (1) elsewhere. Briefly, the method consists of estimation of seasonal change and linear secular trend from data for previous years and projection of the curves for appraisal of current levels.

In figure 10 regional differences in the 1957–1958 and 1959–1960 outbreaks may be observed. In general the bi-modal pattern of the 1957–1958 outbreak was characteristic of divisions east of the Rocky Mountains, except for New England and—to a lesser extent—the Middle Atlantic division, in which the second wave of mortality was less pronounced. This was also true of the Pacific and Mountain states. In the Pacific states even the first wave of the epidemic caused only moderate excess mortality.

In 1959–1960, however, both the Pacific and Mountain states experienced a high excess mortality. The 1959–1960 experience was similar in all divisions except the Middle Atlantic in which excess mortality was very small.

MORTALITY IN THE TOTAL POPULATION OF THE UNITED STATES

The mortality data collected from cities were not suitable for calculating mortality rates because they were not only preliminary in nature but also included varying and unknown proportions of nonresident deaths. More suitable mortality data for estimating rates became available from a 10 per cent sample of death certificates submitted every month by each of the states. These also showed a well-defined rise in mortality

[1] From the National Office of Vital Statistics, and Statistics Section, Epidemiology Branch, Communicable Disease Center, U. S. Public Health Service, United States Department of Health, Education, and Welfare, Washington, D. C.

from influenza and pneumonia in the period from October, 1957, to March 1958, inclusive. It was also apparent from these figures that there was a coincident rise in mortality from some other causes, principally cardiovascular diseases. Since final figures on all deaths occurring in the epidemic period are now available and are corrected for residence, they will be used in the following discussion on mortality in the 1957–1958 epidemic. Final data for prior years will be used for comparison.

Mortality in 1957–1958 Epidemic Compared with Some Prior Years

Some concern was expressed before the nation-wide epidemic began in the fall of 1957 that there might be a repetition of the high mortality in the 1918–1919 influenza epidemic because antibodies against the new "Asian" strain were absent in the younger age groups in whom incidence is always highest. However, this proved to be an unfounded fear as shown in figure 11. This chart shows mortality by months during several epidemic periods in the past decade, including the 1957–1958 episode. The peak of mortality reached in March, 1950 and 1951, was only slightly lower and the peak reached in February, 1953, was higher than that of November, 1957. However, the period during which mortality remained at a relatively high level in each of the years 1950, 1951, and 1953 was very much shorter than the period of high mortality in the 1957–1958 epidemic. In these earlier years there was a rapid rise to a peak and an equally abrupt decline. The relatively long period of high mortality in 1957–1958—approximately six months—was quite unusual, and in this respect it did resemble the 1918–1919 epidemic. No others, including the relatively extensive epidemic of 1891–1892, showed this biphasic type of occurrence.

Seasonal Changes in Mortality

It may be seen in figure 12 that, with the rise in mortality from influenza and pneumonia in the winter months, there was a coincident rise in the death rate from cardiovascular diseases. Note that the scale for cardiovascular diseases in figure 12 is only one-tenth the scale for pneumonia-

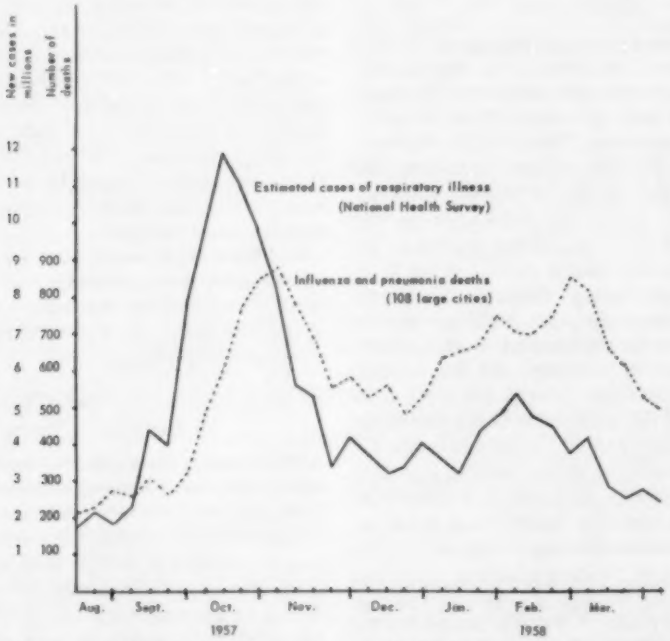


Fig. 9. New cases of respiratory illnesses compared with influenza and pneumonia deaths, reported by week.

influenza. This has occurred in non-epidemic as well as epidemic years for a considerable period of time. When there was a sustained rise in influenza and pneumonia, as in 1957–1958, there was a similar sustained rise in the number of deaths from cardiovascular disease. The reason for this concurrent rise is not clear, but it is quite possible that influenza and other viral respiratory infections were contributing causes, and in many instances they probably were not recognized or did not appear to be important enough to warrant their inclusion as contributing causes of death.

Excess Mortality

Collins' studies over the years have clearly shown (figure 13) the excess pneumonia-influenza mortality associated with influenza epidemics, but have also demonstrated (2) the associated excess mortality from other causes. Since 1918–1919, when 92 per cent of the excess was due to influenza and pneumonia, a gradually decreasing amount of the excess mortality has been due to this cause and a corresponding increase in other causes, particularly in cardiovascular diseases. As shown in figure 14 the excess mortality credited to influenza and pneumonia has not been more than 20 to 25 per cent of the total in epidemics of the past ten to fifteen years.

An estimate of excess mortality by age and selected causes in the 1957–1958 and 1960 epidemics is provided in table 1. The number of excess deaths shown in each category of table 1 is the difference between the observed number and an expected number computed (1) by projection of the linear secular trend during preceding non-epidemic years.

A total of more than 80,000 excess deaths occurred in the two epidemics, with nearly half of them in the first three-month period of the 1957–1958 epidemic. During this period, 43 per cent of excess deaths were among persons less than 65 years of age in comparison with 32 per cent in January through March, 1958, and 20 per cent in January through March, 1960. The latter percentage was further reduced by the deficit in expected deaths of those less than one year of age.

By cause of death, cardiovascular-renal diseases accounted for somewhat less than 50 per cent of the excess mortality in October–December, 1957, and in January–March, 1960, but in the January–March "second wave" of the 1957–1958 epidemic, 65 per cent of the excess mortality was due to cardiovascular-renal diseases.

Age Distribution of Influenza and Pneumonia Mortality

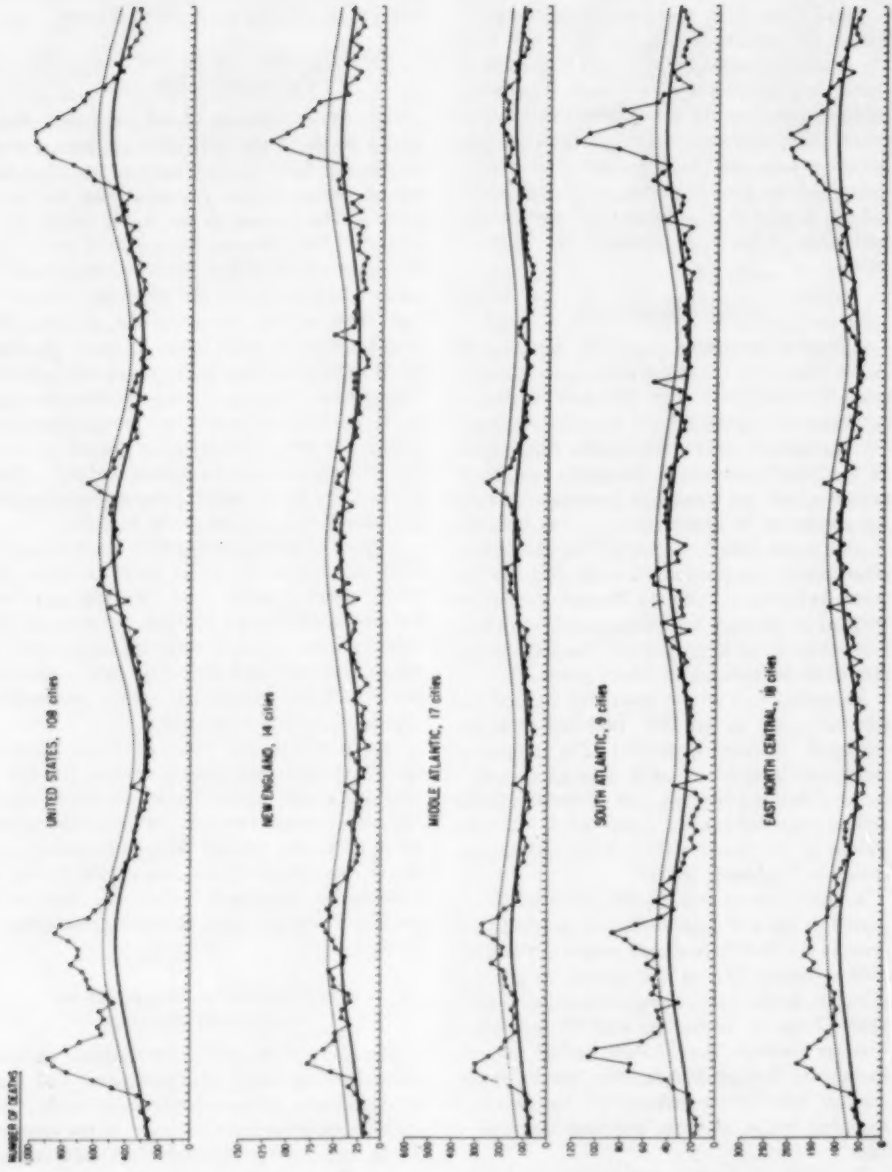
The age distribution of influenza and pneumonia deaths in the 1957–1958 epidemic period is shown in figure 15 with rates of the same six-month period of the previous year for comparison. The contour of the curve, which is U-shaped, is not different from that of other epidemic periods of recent years, i.e., high in very young children and in the older age groups. It had none of the characteristics of the "W" type of curve of 1918 shown in figure 16 when death rates were high in the young adult group. Nevertheless, relative excess mortality was high in the thirty to thirty-nine age group (figure 17) during the October–December period of 1957 and moderately high in January–March, 1958. In the latter period relative excess mortality was moderately high at ages thirty to sixty.

A more detailed presentation of pneumonia-influenza deaths by age is given in figure 18, which shows monthly data for 1956 and 1957 for several age groups. In 1956, the seasonal distribution was similar for all age groups, low in the summer and high during the winter months, but in 1957 the pattern was greatly changed by the Asian influenza epidemic.

In the groups less than forty-five years of age, pneumonia-influenza mortality reached a peak in October except for infant deaths which increased through December in the usual seasonal pattern. Among persons forty-five years of age and older, deaths were above 1956 levels in October but continued to increase, reaching a peak in November, then decreasing somewhat in December.

Differences in Mortality by Geographic Division

The effect of the 1957–1958 epidemic on mortality from influenza and pneumonia and from cardiovascular diseases is shown in table 2 for each of the geographic divisions of the country. These data are for calendar years only and are not available at this time for 1958. The percentage increase in mortality rates from influenza and pneumonia in 1957, compared with those for 1956, varied from about 20 to 40 per cent. The


NUMBER OF DEATHS
UNITED STATES, 108 cities
NEW ENGLAND, 14 cities
MIDDLE ATLANTIC, 17 cities
SOUTH ATLANTIC, 9 cities
EAST NORTH CENTRAL, 18 cities

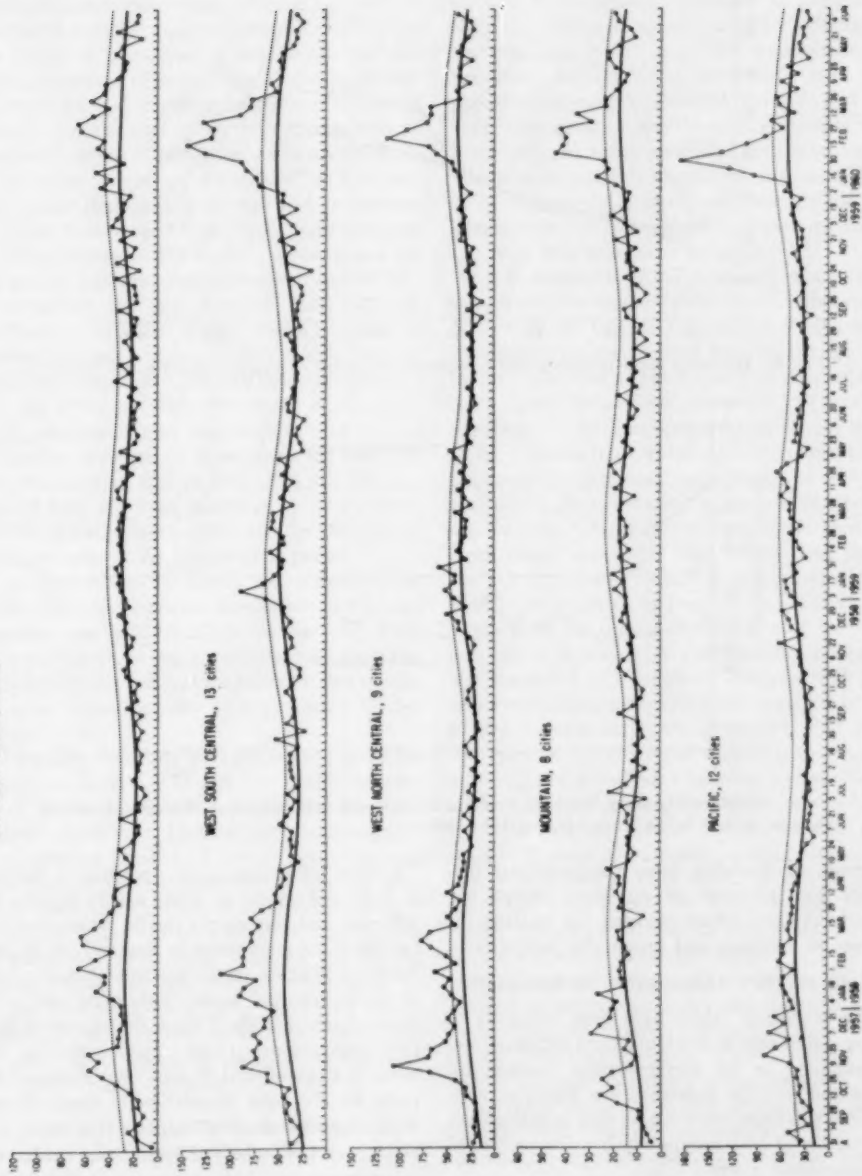


FIG. 10. Weekly pneumonia and influenza deaths, 1957–1960.

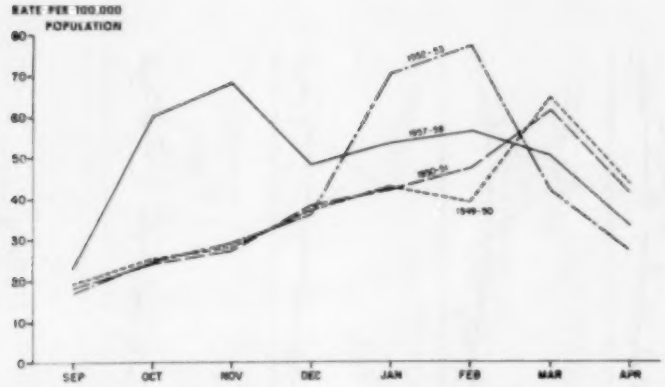


FIG. 11. Mortality from influenza and pneumonia for selected years in the United States.

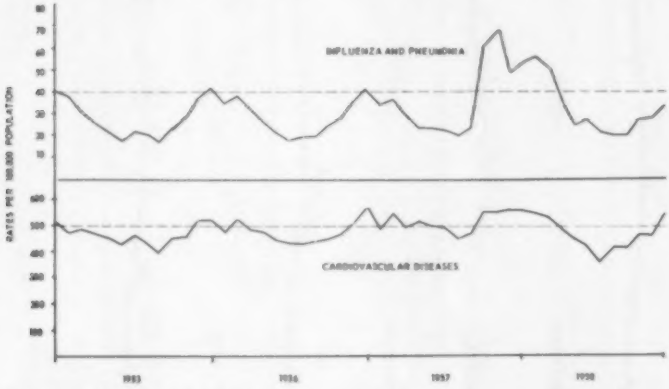


FIG. 12. Mortality from influenza and pneumonia and cardiovascular diseases on annual basis by month in the United States, 1955–1958.

increase in mortality from cardiovascular diseases was apparent in all areas except the Pacific division which reported the smallest increase in influenza and pneumonia mortality.

RACE AND SEX DIFFERENCES IN MORTALITY

No statistical tables have been prepared to show differences in mortality from influenza and pneumonia or for cardiovascular diseases by race and sex. The mortality was higher in nonwhites and there was a higher rate in males than in females of both groups which is also present in non-epidemic years.

MORTALITY IN OTHER COUNTRIES

A few data are available which make it possible to compare the effects of the 1957–1958 epidemic on mortality in different countries.

During the course of the epidemic in the fall of 1957 and winter of 1958, weekly reports on influenza and pneumonia deaths were available for 160 of the large towns in England and Wales. As shown in figure 19, the rise and fall in numbers of deaths reported weekly were quite similar to those observed in the United States except that they occurred about one month earlier in the cities in England and Wales. Vital statistics reports for the total population of England and Wales for 1956 and 1957 indicate that there was no increase in numbers of deaths from cardiovascular diseases, as there was in the United States. While the death rate from influenza and pneumonia was higher than in the previous three years, it did not equal that of 1951, when Liverpool was affected so severely.

Wide differences are normally to be found in influenza and pneumonia mortality rates reported in various countries, some of which may be caused by variations in social conditions and age composition of population. The completeness in registration of deaths and customs in certifying causes of death may affect the comparability of figures in different countries. Data for 28 countries (table 3) which are available for 1956 and 1957 show that about one third had an increase of 30 per cent or more in mortality from influenza and pneumonia. A few had no increases and the remainder had moderate increases in mortality.

Zhdanhov (3) has reported that influenza mortality in 310 cities of the Soviet Union showed a sharp rise in October and November. This rise was from about 3 to 7 per 100,000 population and did not affect the downward trend of mortality from pneumonia that had been in progress for a number of years. In these cities the rates for pneumonia were 60.4 in 1955, 50.8 in 1956, and 45.5 in 1957. However, mortality for cardiovascular diseases rose 23.8 per cent and for tuberculosis 46.1 per cent during the epidemic period.

Another report by Raska and associates (4) stated that the influenza death rate in Czechoslovakia remained relatively low in 1957. They showed that there was a concurrent seasonal rise in mortality for respiratory infection and cardiovascular diseases similar to that for the United States.

Mortality statistics for 1959 are not available for other countries except for weekly reports from England and Wales during the first four months of the year. These reports indicated that a substantial number of deaths from influenza and pneumonia were occurring during the time when epidemics caused by A2 and B viruses were occurring. There was little demonstrable increase in mortality in the large cities of the United States during this same period, when most localized outbreaks were being confirmed as influenza, type B. A few localized outbreaks due to influenza A2 were also reported, the largest of which occurred in California.

Bacterial Complications of Influenza, 1957–1958

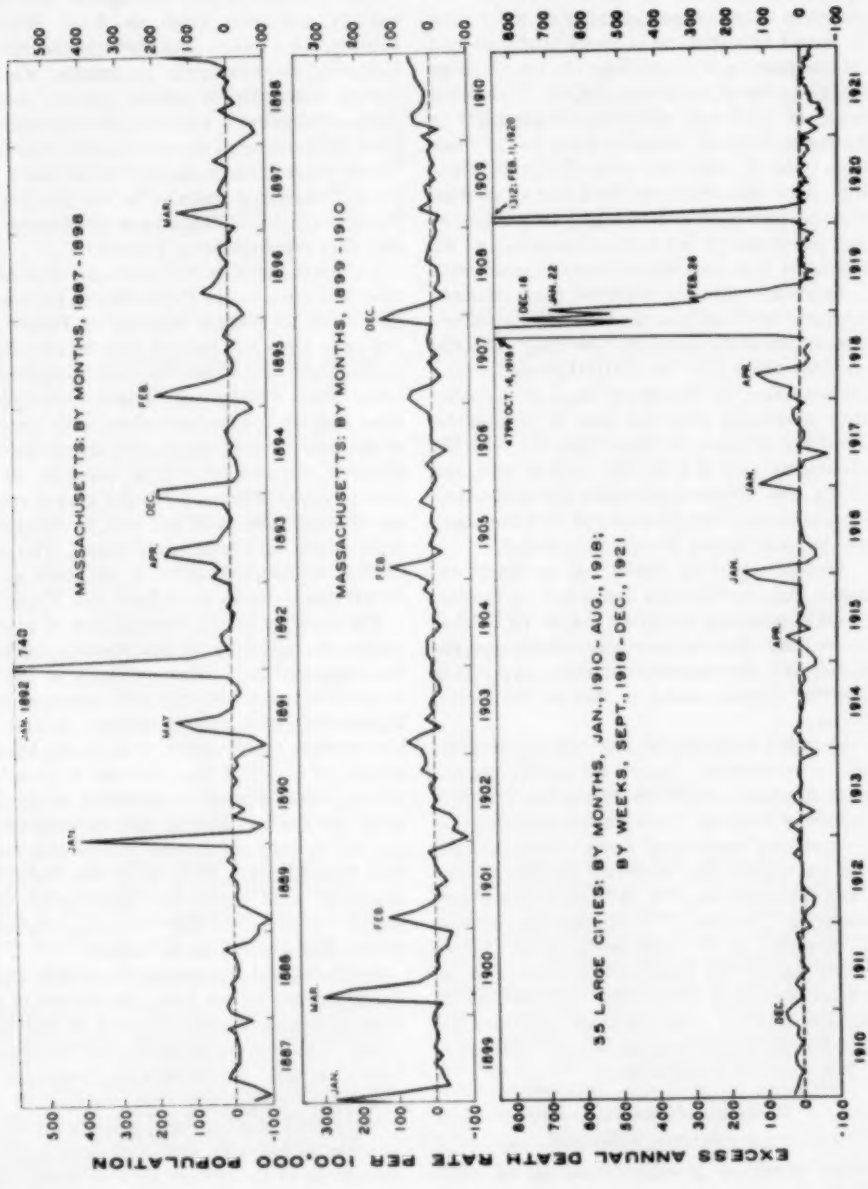
Our published mortality statistics for 1957–1958, or for any other epidemic year, do not contain information on the frequency of the various types of bacterial agents that caused pulmonary complications even though the etiologic type of pneumonia may be specified on some death certificates. Unfortunately, pneumonias are classified by anatomic types in *Vital Statistics Reports*, i.e., lobar- and bronchopneumonias. However, staphylococcal pneumonia was reported frequently in clinical studies, and in some communities it was the most commonly isolated bacterial agent associated with fatal cases. On the other hand, in one city it was found that 24 of 38 bacterial pneumonias complicating influenza were due to *Diplococcus pneumoniae* and only 6 to *Staphylococcus pyogenes*.

An investigation of 477 fatal and 43 nonfatal cases was made by the Public Health Laboratory Service of the British Ministry of Health (5). Influenza virus was isolated from 80 per cent of patients who died within five days of onset of the acute illness. *Staphylococcus aureus* was isolated from lung or sputum specimens of 62 per cent of the patients from whom such specimens were obtained. Staphylococci were found in 49 per cent of young children, in 88 per cent of school-age children, and in 31 per cent of old persons from whom specimens were taken. The cases studied represented about 3 per cent of the 16,000 excess deaths in England and Wales.

The need for careful investigation of cases of pneumonia following or complicating influenza was suggested in a follow-up study of 140 patients with pneumonia who were admitted to an English hospital during the epidemic in 1957 (6). Thirty-eight were infected with *Staphylococcus aureus*, 13 of whom were believed to have been infected with drug-resistant strains in the hospital. In Boston, Martin and co-workers (7) studied 20 cases of bacterial pneumonias associated with influenza, 11 of which were fatal, and nearly all were caused by staphylococci. In 5 cases it was suspected that the staphylococcal infection was acquired in the hospital.

Staphylococcal pneumonia secondary to influenzal infections has been encountered in previous epidemics. Finland reported 66 such cases in the 1940–1941 epidemic. These bacterial invaders are now considered more dangerous because many strains are drug resistant. It also appears that treating uncomplicated cases of influenza in hospitals may sometimes be disadvantageous to the patient because of the possibility of acquiring resistant strains in the hospital.

Clinical studies as well as some epidemiologic investigations have indicated that deaths were


EXCESS ANNUAL DEATH RATE PER 100,000 POPULATION
MASSACHUSETTS: BY MONTHS, 1887-1898
MASSACHUSETTS: BY MONTHS, 1899-1910
35 LARGE CITIES: BY MONTHS, JAN., 1910 - AUG., 1918;
BY WEEKS, SEPT., 1918 - DEC., 1921
JAN. 1892
740
JAN.
MAY
APR.
DEC.
FEB.
MAR.
MAR.
JAN.
FEB.
DEC.
DEC. 18
JAN. 22
FEB. 26
4799 OCT. 4, 1918
3312 FEB. 11, 1920
1887
1888
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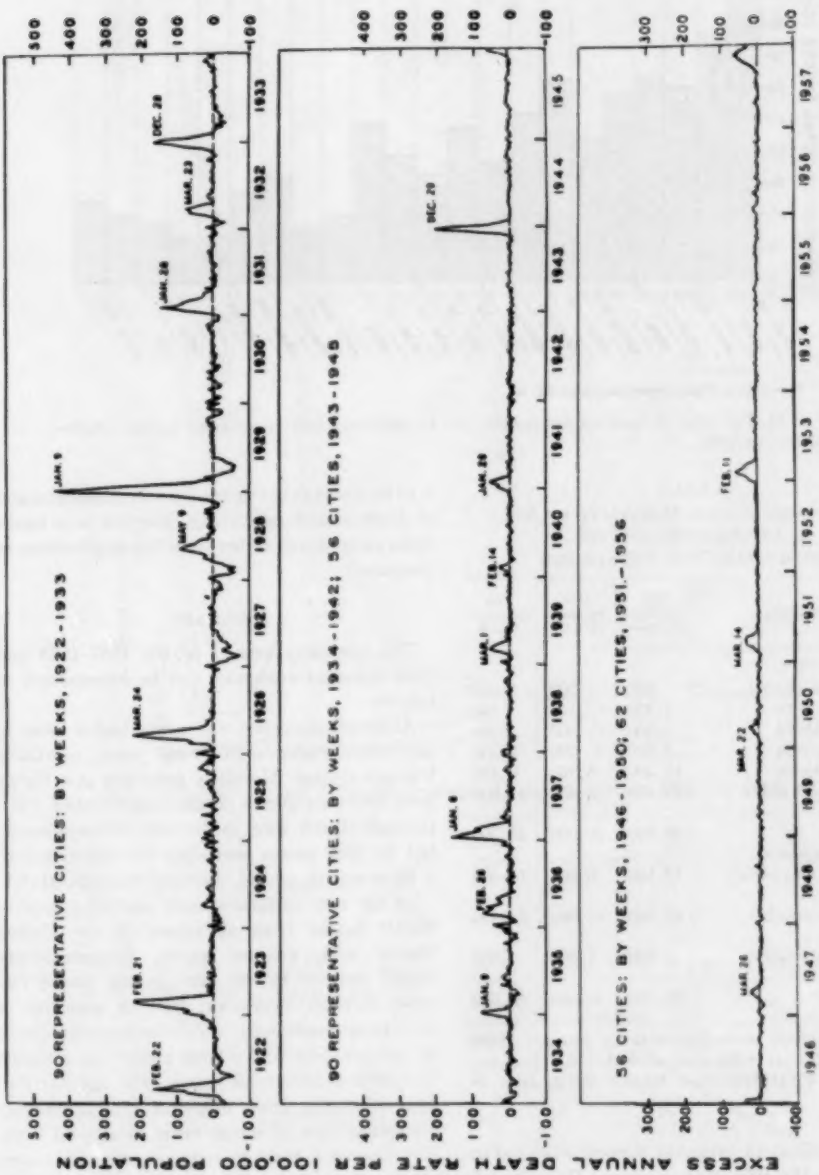
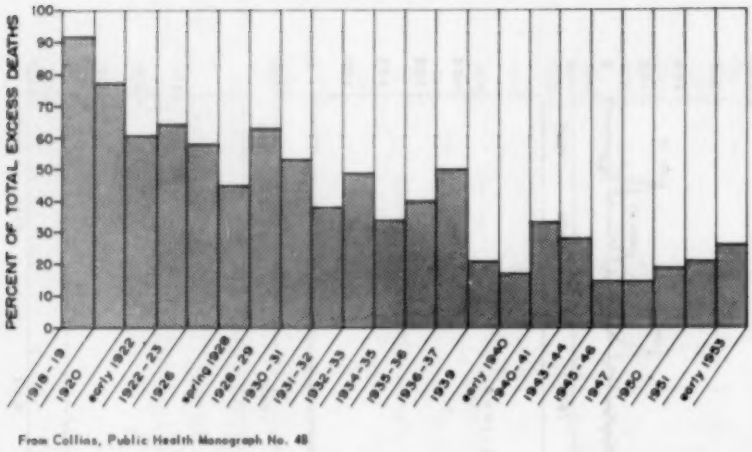

EXCESS ANNUAL DEATH RATE PER 100,000 POPULATION
90 REPRESENTATIVE CITIES: BY WEEKS, 1922-1933
90 REPRESENTATIVE CITIES: BY WEEKS, 1934-1942; 56 CITIES, 1943-1945
56 CITIES: BY WEEKS, 1946-1950; 62 CITIES, 1951-1956

From Collins, Public Health Monograph No. 48

FIG. 13. Excess annual death rate in Massachusetts and in representative United States cities, 1887–1957.



From Collins, Public Health Monograph No. 48

FIG. 14. Per cent of total excess deaths due to influenza and pneumonia during selected epidemic periods.

TABLE 1

ESTIMATED EXCESS MORTALITY BY AGE AND SELECTED CAUSES

United States, 1957–1958 and 1960

Age Group	1957 October–December	1958 January–March	1960 January–March
years			
Less than 1	600	200	−3,200*
1–14	1,300	100	100
15–24	1,000	300	200
25–44	3,600	700	2,200
45–64	10,400	5,300	6,100
65 and older	22,400	13,400	21,300
All ages	39,300	20,000	26,700
Selected causes			
Pneumonia-influenza	12,100	6,000	10,600
Cardiovascular-renal	18,700	13,000	12,200
All other causes	8,500	1,000	3,900
Total	39,300	20,000	26,700

* The infant mortality rate in January, 1960, was the lowest rate ever recorded for January. Monthly Vital Statistics Report 9(3)2, May 18, 1960.

not infrequent in pregnant women who had influenza in the 1957–1958 epidemic. However, the maternal mortality rates in this period were no higher than in the recent non-epidemic years. The explanation for this apparent contradiction is to be found in the method used in coding causes of death in such individuals. The rule is to credit them to influenza rather than to complications of pregnancy.

SUMMARY

The mortality aspects of the 1957–1958 and 1960 influenza epidemics can be summarized as follows:

Although incidence was much higher than in any other epidemic of recent years, mortality was not greater. Mortality remained at a higher level over a six-month period from October, 1957, through March, 1958, in the 1957–1958 epidemic; but in 1960 excess mortality was restricted to a three-month period, January through March.

In the two epidemics there was an excess of 86,000 deaths from all causes in the United States, with greatest excess (approximately 40,000 deaths) in the three-month period October through December, 1957. A majority of the excess deaths were in persons sixty-five years of age and older with a large proportion assigned to cardiovascular-renal causes. The age distribution of deaths from influenza and pneumonia resembled that of recent years in general form, but showed a marked relative excess in young adults during October through December of 1957.

An increase in influenza and pneumonia mortality was evident in most countries for which

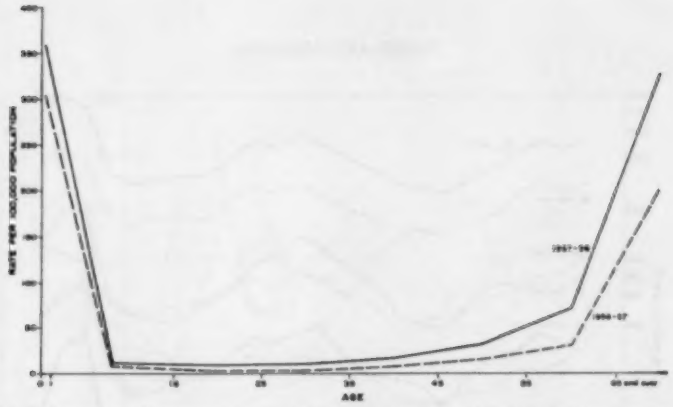


FIG. 15. Influenza and pneumonia mortality rates in the United States by age, October–March, 1956–1957 and 1957–1958.

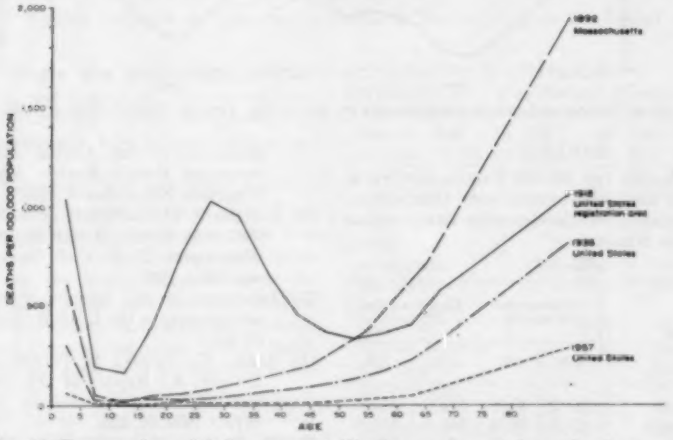


FIG. 16. Pneumonia and influenza mortality by age in certain epidemic years.

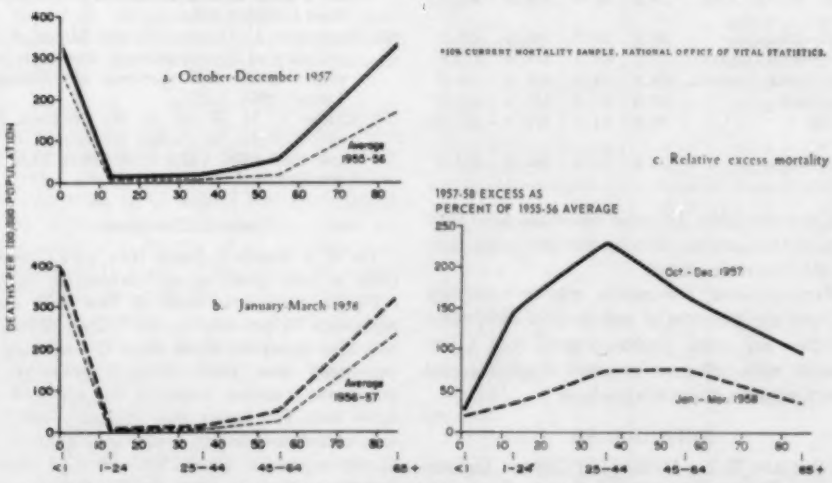


FIG. 17. Excess pneumonia and influenza mortality by age, United States, October–December, 1957, and January–March, 1958.

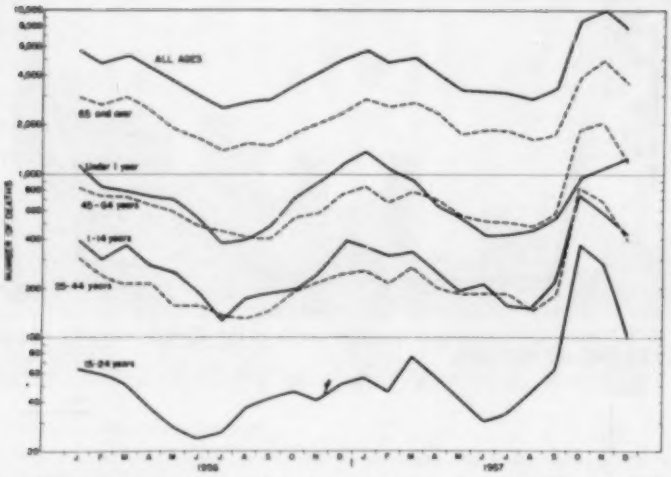


FIG. 18. Influenza and pneumonia deaths by broad age groups, United States, 1956–1957.

TABLE 2

MORTALITY RATES PER 100,000 POPULATION FROM INFLUENZA AND PNEUMONIA AND CARDIOVASCULAR DISEASES BY GEOGRAPHIC DIVISION OF THE UNITED STATES, 1956–1957

Division	Influenza and Pneumonia		Cardiovascular Diseases	
	1956	1957	1956	1957
New England	29.2	35.6	604.2	612.3
Middle Atlantic	27.8	36.1	586.0	599.4
East North Central	28.0	34.5	522.8	538.6
West North Central	26.3	37.8	529.9	554.1
South Atlantic	28.2	36.7	441.4	455.5
East South Central	31.2	38.4	448.4	472.9
West South Central	28.6	35.4	407.2	415.8
Mountain	32.0	41.6	357.8	373.5
Pacific	26.5	31.6	478.1	477.3
United States	28.2	35.8	501.5	514.9

data are available. In some countries mortality from cardiovascular diseases also increased during the epidemic period.

Staphylococcal pneumonia was a relatively common complication of influenza in some communities, and some studies suggest that a few patients with influenza acquired staphylococcal infections after being hospitalized.

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GENERAL DISCUSSION

DR. R. F. KORNS (Albany, New York): Actually there is little point to my elaborating in detail our experience in the State of New York since it represents 10 per cent of the United States, and you have heard the whole story this morning. Our experience with Asian influenza in many ways represents a mirror image of the whole. I must agree with Dr. Dauer that mortality trends are very useful and readily available as a direct index of the impact of the epidemic wave. I might illustrate, though, to carry Dr. Langmuir's remarks

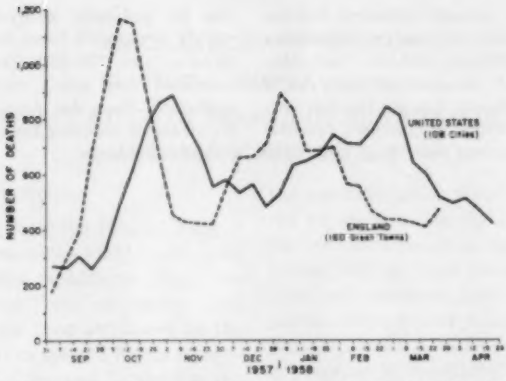

FIG. 19. Weekly influenza and pneumonia deaths in England and in the United States, 1957–1958.

a bit further within the local setting, certain vagaries in the distribution of the disease and spread. One can illustrate on the state level, just as he did internationally, how influenza strikes certain areas first.

Dr. Morris Greenberg, who was with us today, has shown that the crowded parts of New York City, the upper east side Harlem area, was first involved. Likewise in different boroughs, Asian influenza hit first the largely crowded areas occupied by the lower social and economic groups. From these areas it spread in a pseudopod manner to other parts of the city and state. Staten Island, which is isolated by water, was hit late and much less intensely. This sort of pattern was repeated in other cities and explains the continuation of Asian influenza with its high mortality over at least a six-month period. Certain areas of the population were missed in the initial epidemic wave presumably, and the elderly debilitated people possibly did not achieve effective contact with the disease earlier.

The one remark I would like to make applies to the importance of the role of "shoeleather" type of epidemiology or data-collecting process at the local level in a state or county health department. This leaves much to be desired but, nevertheless, it is one of the important mechanical means for keeping aware of morbidity of disease present in the population. It is one of the means of gathering the indices to which Dr. Langmuir referred. Unfortunately, however, we in a health department often hear first of the beginning of influenza outbreaks through the newspapers and other communication media. This is a common experience everywhere, I am sure.

However, setting up a routine mechanism of keeping continuous surveillance on school absenteeism, industrial absenteeism, hospital admissions

TABLE 3

INFLUENZA AND PNEUMONIA MORTALITY RATES PER 100,000 POPULATION ON SPECIFIED COUNTRIES, 1956 AND 1957, AND PERCENTAGE INCREASE IN MORTALITY FROM 1956 TO 1957

Countries	1956	1957	Percent Increase
Africa			
Mauritius	58.3	106.3	82.3
Rhodesia	25.3	24.9	−1.5
The Americas			
Canada	35.9	43.1	20.0
United States	27.0	35.8	32.6
Costa Rica	56.2	94.7	68.5
Guatemala	211.1	276.2	30.8
Trinidad	52.7	69.1	31.3
British Guiana	60.5	65.7	8.5
Colombia	93.1	110.6	18.8
Asia			
Taiwan	86.1	108.3	26.0
Hong Kong	146.3	150.6	2.9
Japan	34.0	51.6	51.7
Portuguese India	65.4	77.9	19.1
Israel	25.5	29.6	16.0
Europe			
England and Wales	56.0	65.8	17.5
Scotland	42.3	52.9	25.0
Finland	32.9	73.6	123.7
Austria	56.7	73.7	30.0
France	60.0	73.1	21.8
Hungary	74.0	90.5	20.3
Ireland	47.0	58.8	25.1
North Ireland	48.0	62.1	29.4
Luxembourg	47.4	61.4	29.5
Netherlands	29.4	30.2	2.7
Portugal	99.2	99.7	.5
Malta	31.2	23.6	−24.3
Iceland	47.0	58.8	25.1
Oceania			
Australia	34.9	38.3	9.7

Source: Demographic Yearbook, Statistical Office of the United Nations, New York.

for respiratory disease, patient treatment indices, et cetera, will yield early information concerning the existence of an influenza problem. Our state and certain city health departments carry on a serologic type of surveillance. You are familiar with Widelock's reports in which he sampled prenatal Wasserman blood specimens from New York City also for influenza antibodies on a continuous weekly or monthly basis. His findings, certainly in terms of the 1957–1958 Asian influenza epidemic, correlated very nicely with the observations on morbidity. Thus, this is yet another crude but useful means of assessing the initial appearance of an influenza epidemic.

MECHANISMS OF SPREAD

CLAYTON G. LOOSLI, *Chairman*

THE MECHANISM OF SPREAD OF ASIAN INFLUENZA[1]

WILLIAM S. JORDAN, JR.

Introduction

Asian influenza was truly the Phineas Fogg of diseases, spreading around the world in less than twice eighty days with a rapidity to delight the most sanguine of Jules Verne virologists. This global dissemination has been attributed to the capacity of Asian virus to infect a highly susceptible population (1, 2). Beyond belaboring the obvious, what more needs to be said about how Asian influenza spread?

Students of past epidemics and pandemics have noted that clinical influenza spread rapidly even before the advent of fast transportation (3) and that dates of maximal incidence did not provide good evidence of routes of spread (4). Such discrepancies spoiled the case-to-case transfer explanation, and prompted several suggestions as to how a new antigenic strain of virus could appear within a short space of time in widely separated countries: (*1*) mutation on a single occasion followed by rapid spread; (*2*) widespread preseeding of virus in masked form, with its almost simultaneous provocation to infectivity (3); (*3*) simultaneous mutations in multiple foci (5). The abundance of data collected as Asian virus extended throughout the world in 1957 established direct lines of transmission from one focus to another, and makes it unnecessary to invoke multiple mutations to explain pandemic occurrence (6). Do the data also disprove the suggestion regarding pre-seeding of virus and provocation to infectivity? Not necessarily. On the contrary, the behavior of Asian influenza lends support to this concept.

How else can we explain the fact that, after the introduction and multiple seeding of virus in the United States during the summer, outbreaks were consistently limited to situations in which large numbers of people were crowded together (7)? How else can we explain the almost simultaneous outbreaks in the fall in Arizona, Cleveland, Oregon, Florida, and New York? These are some of the questions which merit discussion. What part did exposure within the school play in the dissemination of virus? In the United States (8), in Alaska (9), in Great Britain (10, 11), in Japan (12), and elsewhere, epidemic influenza first appeared among school children and then spread to preschool children and adults. The first community spread in the United States was associated with a July opening of schools in Louisiana (13, 14). In Liverpool, independent schools started their terms three weeks later than public schools, and their epidemic peak also occurred three weeks later (11). The high attack rate consistently noted in the five- to nineteen-year-olds has been attributed by some (12) to the high rate of exposure in schools rather than to greater susceptibility. Conversely, it has been suggested that the low attack rate among older people may have been due to lack of contact with school children (10) rather than to greater resistance attributable to specific antibodies (2, 15).

What part did exposure within the family play in the dissemination of virus? In some outbreaks (14, 16, 17), attack rates increased with increase of family size; in others (18), differences in family size had no effect. Although the data are not detailed enough for analysis, it is suggested that the observed attack rates could have been as much a function of the age of the children in the family as of the number of individuals. What evidence is there for intra-familial spread? After studying an earlier epidemic, that due to A1 virus in 1951, Hope Simpson (4) noted the paradox of an "epidemic of a disease with a very high attack rate and against which there appears to be little immunity in the population, which the school child does not bring home from school and which does not appear to be transmitted inside the home." He made this observation because first cases in families and subsequent cases showed no special age preference, and because, in an analysis of intervals from the first case, susceptible persons fell rapidly and steadily away from the zero day. What did we learn about first cases and intervals during the Asian influenza epidemic?

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TABLE 1

EXAMPLES OF SEEDING OF ASIAN INFLUENZA VIRUS IN 1957

Location	First Isolation	Epidemic Peak
Louisiana (14)*	Late June	August 5–11
Liverpool (11)	June 27	September 27–28
Cleveland (19)	June 24	October 13–19
New York (25)	August 6	October 4–11

* Numbers in parentheses indicate references.

CLEVELAND FAMILY STUDY

Answers to this and to the preceding questions may be sought by examination of data derived from study of a population of families in Cleveland (19).[2]

This population had been under observation for nine years, with particular emphasis on respiratory disease, and mechanisms had been established for the reporting and recording of illnesses and for the collection of specimens for laboratory diagnosis. Serum specimens had been obtained from all family members in the spring of 1957. The adults were bled again early in September. Beginning on September 22, the staff of the Department of Preventive Medicine at Western Reserve University collected pharyngeal swabs from all of those with reported illnesses, and classified each according to broad diagnostic categories. After the epidemic, serum specimens were collected from both adults and children.

Asian influenza virus was first isolated in Cleveland in June, and two other sporadic infections were documented in July and August. The first known case in the family study population occurred on September 2; the next two on September 19 and 20 at the beginning of the epidemic. Such a long interval between introduction and epidemic was the rule in the temperate zone. Examples are listed in table 1. What was the virus doing all of this time? Epidemics developed rapidly when the virus was introduced into equatorial countries; introduction into Europe, North America, or South America did not, with few exceptions, result in epidemics until the fall-winter season (8, 20).

Cleveland schools opened on September 4. By September 22, the beginning of week 1 in figure 1, respiratory disease rates were already high. Most of these illnesses resembled the common cold. Influenza-like illnesses then increased in frequency, reaching a peak during the week of October 13. In eight weeks, the epidemic was over. During the ten weeks of clinical observation, the distribution of illness by broad diagnostic categories (table 2) resembled the stable figures for this population in past years. Respiratory infections accounted for two-thirds of all illnesses; 13 per cent were classified as influenza-like. Seventy-three per cent of all persons with respiratory illnesses were tested for virus; Asian virus was recovered from 43 per cent. Virus was isolated from 92 per cent of those thought clinically to have influenza, and from 27 per cent of those with milder illnesses classified as common respiratory disease.

Virus was isolated from fifty-two, or 85 per cent, of the sixty families under observation, and from 126, or 41 per cent, of the 308 members of the families. When the pre- and post-epidemic sera were tested simultaneously, 93 per cent of persons from whom virus had been isolated showed fourfold or greater increases in antibody titer. In addition to those from whom virus was isolated, the complement-fixation and hemagglutination-inhibition tests detected significant rises in 20 persons and in two more families. Thus, the laboratory demonstrated that 146 adults and children, or 47.4 per cent of the study population, were infected during the epidemic. When the 110 persons given various amounts of vaccine are excluded from the analysis, the attack rate shown by the serologic procedures is 55 per cent.

Whether measured by virus isolation or antibody increase, the attack rate was highest in the ten- to fourteen-year-olds, although all children from five through the late teens had generally high rates (table 3). The rates in the parents, most of whom were in their thirties or early forties, were much lower. This is the pattern reported by many studies (2, 6, 10, 12, 14, 17, 20–23). With few exceptions, such as those reported for citizens of Melbourne (24) and Aleutian natives (25), attack rates were highest in the five- to nineteen-year-olds and decreased thereafter with increasing age. Obviously, therefore, the more children in a family, the higher the attack rate would be.

The rate pattern within the family resembled that for the non-influenzal common respiratory diseases (26), suggesting that certain similar unknown factors, apart from the possession of antibodies, may operate to influence the attack rates

[2] The Cleveland investigation was conducted under the sponsorship of the Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board, and was supported in part by the Office of the Surgeon General, Department of the Army.

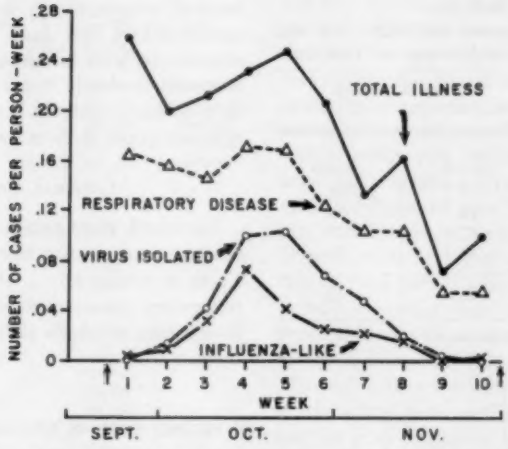

FIG. 1. Incidence of illness and virus isolations by week; population of Cleveland families, September 22–November 30, 1957.

of many viral respiratory infections. When examined by status in the family (table 4), influenza attack rates were highest in the school children and lowest in the fathers. The importance of the school is emphasized further by the family study data. Considering the first virus-positive case as the index case, school children were responsible for introducing virus into the home in 83 per cent of the family episodes. Despite their daily contacts, fathers introduced virus only twice.

TABLE 2

CLASSIFICATION OF ILLNESSES AND ISOLATION OF INFLUENZA VIRUS, CLEVELAND FAMILY STUDY
September 22–November 30, 1957

	Total Illnesses		Isolation of Virus	
	Number	Per Cent	Per Cent Tested	Per Cent Positive
Common respiratory disease	314	55	67	27
Influenza-like	71	13	100	92
Specific respiratory disease	4	1	75	0
Total respiratory disease	389	68	73	43
Gastroenteritis	100*	18	29	17
All other	81	14	14	0
Total	570	100	57	39

* Ten gastroenteritis illnesses had simultaneous onsets with respiratory illnesses.

TABLE 3

INFLUENZA ATTACK RATES BY AGE IN MEMBERS OF CLEVELAND FAMILIES

Age Group	Measure of Infection	
	Virus Isolation	Antibody Increase*
years	*per cent*	*per cent*
0–4	43	50
5–9	58	67
10–14	59	78
15+	47	71
Adults	19	24
Total	41	55

* In unvaccinated persons.

Once introduced into the home, how well did the virus spread? As measured by virus isolation, the secondary attack rate was 35 per cent. As measured by serologic tests, the secondary attack rate in twenty-one unvaccinated families was 36.5 per cent. But the length of the intervals between the onsets of virus-positive cases suggested that many of the secondary infections were not acquired in the home (figure 2). Multiple isolations were obtained in thirty-eight families. In these, twenty of the thirty-eight intervals between first and second cases (indicated by the shaded blocks) were six days or longer, the longest being twenty-six days. The median interval between the first and all subsequent cases was 7.4 days. Similar observations have been recorded

TABLE 4

INFLUENZA ATTACK RATES BY STATUS IN THE FAMILY IN MEMBERS OF CLEVELAND FAMILIES

Status in the Family	Measure of Infection	
	Virus Isolation	Antibody Increase*
	per cent	*per cent*
Father	13	16
Mother	24	32
School child	57	73
Preschool child	44	30
Total	41	55

* In unvaccinated persons.

dence of intra-family spread. Although the preponderance of first cases, and presumably introductions, were in school children, we cannot demonstrate clearly that these children infected their siblings or parents. Nor do we know why the epidemic began in the schools.

GENERAL COMMENT

Are schools more crowded than homes? Is person-to-person contact more intimate in school? There is consistently a sharp rise in common respiratory disease rates within two weeks after the opening of schools (26). To explain this phenomenon, it has been assumed that a number of agents were already seeded in the population, and that widespread dissemination was favored by multiple contacts at school. The behavior of Asian virus in the United States in the summer

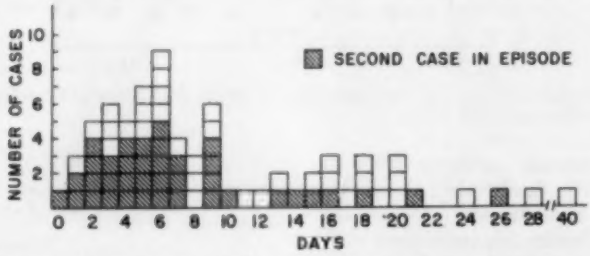

FIG. 2. Intervals between onsets of first and subsequent virus-positive cases of influenza in Cleveland families (19).

TABLE 5

MEDIAN INTERVAL BETWEEN FIRST AND SUBSEQUENT CASES OF ASIAN INFLUENZA IN FAMILIES

Study Area	Interval
	days
"Lavender City, North State" (17)*	6.7
"Grayville, South State" (17)	6.9
Cleveland, Ohio (19)	7.4

* Numbers in parentheses indicate references.

by Drachman, Hochbaum, and Rosenstock (17) for outbreaks in two cities identified as "Lavender City, North State," and "Grayville, South State" (table 5). The median intervals were 6.7 and 6.9 days, respectively. No peak occurred at the expected incubation period when secondary cases in families were plotted by intervals from the index case. Like the Cleveland investigators, Drachman and associates were unable to account for intervals so much longer than the demonstrated incubation period (27). Like Hope Simpson (4), they commented on the paradox of greater risk of infection in larger families without clear evi-

TABLE 6

ASIAN INFLUENZA ATTACK RATES IN "CLOSED" POPULATIONS

Location	Measure of Infection	
	Illness	Antibody
	per cent	*per cent*
Sea transport (29):*		
Crew	6	
Cabin class	16	
Troup class	30	
Ship (AVP) (30)	50	
Medical ward, N. Y. (31)	55	65
State hospital, Calif. (32)	57	70
Training school, Conn. (33)	66	78
Naval station, Calif. (34)	75	76
Alaskan Islands (9):		
St. Paul	82	
Gambell	87	

* Numbers in parentheses indicate references.

TABLE 7

ASIAN INFLUENZA ATTACK RATES IN VARIOUS COMMUNITIES

Location	Measure of Infection	
	Illness	Antibody
	per cent	*per cent*
Singapore (35):*		
European	6	
Asian	29	
Abadan (36):		
Staff	19	
Laborers	33	
Japan (12)	26	
London suburb (22)	25	
London (10)	31	
Louisiana (14):		
White schools	33–50	
Negro schools	48–58	
Cleveland (19)	23	55
"Lavender City" (17)	32	47
"Grayville" (17)	26	38
New York City (25)		35
Melbourne (24)		42
Sheffield (23)		44
Pittsburgh high school (37)		55
New Haven (18)		65

* Numbers in parentheses indicate references.

and fall of 1957 suggests that the same sequence preceded the epidemic occurrence of clinical influenza. The two factors most often considered responsible for the fluctuation of respiratory disease rates are season and crowding. The difference in timing in the northern and southern hemispheres seems to be attributable to season. Zhadanov (28) believes that summer checked the spread of Asian influenza in Russia. As for crowding, attack rates in "closed" populations (table 6) were generally higher than in most communities or even public schools (table 7). Since influenza is spread from person to person, it is reasonable that more contact should mean more spread. Schools mix children from many families. Although intimacy of contact may be no greater in the school than in the home, multiplicity of contact is much greater, and the school must be ranked as the more effective "virus exchanger." Whether a change in the weather or the opening of schools "provokes" influenza virus to infectivity through some mechanism other than crowding is unknown.

Theoretic formulations regarding spread and provocation are presented in figure 3. At some

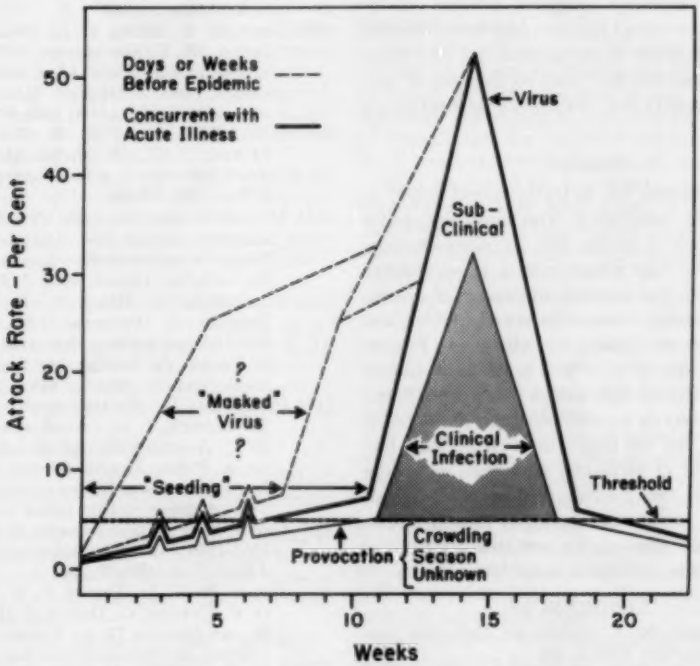

FIG. 3. Theoretic mechanisms of the spread of influenza (*see* text for explanation).

interval—often as long as two to three months—after the introduction of virus into a community or nation, an epidemic of Asian influenza occurred. Virus was isolated from patients with typical illnesses as well as from individuals with milder infections. Prior to the epidemic, only a few sporadic infections were demonstrated in the community or only scattered focal epidemics were recognized in the nation. As more individuals or more communities were infected, the foci coalesced and the epidemic threshold was exceeded. The epidemic flared and was over in eight weeks. Another postulation is that virus was disseminated after introduction, and existed in a "masked" form except for the occurrence of outbreaks provoked by crowding (3). With virus widely seeded, some provocation, such as the opening of schools or fall in temperature, then triggered the epidemic. The available data support only the first formulation, but the epidemiologic discrepancies make the second an intriguing possibility.

Why did the spread of virus stop after only 50 per cent of the population had been infected? Less than one-fourth of adults were attacked, and surely their daily contacts continued. Most likely, the epidemic stopped because three-fourths of the school children had been infected, and the key group as far as both age and exposure are concerned had been so depleted of susceptible members that effective contact no longer occurred.

Summary

Asian influenza did, in truth, spread widely in a susceptible population. The most susceptible group proved to be the five- to nineteen-year-olds, and the high attack rate in these children resulted from the complex interaction of a number of variables: nonspecific susceptibility, lack of protective antibodies, and contact in schools. The school appeared to be a much more important site of virus dissemination than did the home. The long intervals between first and second cases in families, and the longer interval between first introductions of virus and epidemics in nations suggest that the possible seeding of "masked" virus prior to the appearance of clinical disease should be investigated the next time we have an opportunity to anticipate an epidemic.

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General Discussion

Dr. Joseph A. Bell (Bethesda, Maryland): Dr. Jordan has beautifully covered the mechanisms of spread of Asian influenza.

I would like to compare the mechanisms of spread of Asian influenza as he described them with three previous outbreaks—two of type B and one of type A1 influenza—so as to consolidate the picture. First, it is very obvious that during epidemics the virus is spread from person to person and manifests itself as an acute febrile illness. However, we find that we can also isolate the virus in type A and type B outbreaks from people who have only mild so-called common colds without fever. In addition, in a small proportion of cases, we have isolated influenza virus from people who have no illness whatsoever. Therefore, there must be carriers of the disease, which would help to explain, more or less, the data on the chart which Dr. Jordan showed about the endemic spread.

With respect to the period of communicability of the case with Type A1 influenza, we have been able to isolate the virus two days before the onset of illness and for as long as nine days after the onset of illness. Thus, the period of communicability is much longer than we had previously thought.

With respect to the household spread, Dr. Jordan showed a very long period of spread with Asian influenza. In the epidemics of 1952, 1953, and 1955, we found after studying some 400 virus-positive households that 95 to 98 per cent of all subsequent cases from which we could isolate virus occurred within ten to twelve days after the first case. Of course, that is a long time for a highly infectious disease of short incubation period, but it is similar to the long period which Dr. Jordan describes for Asian influenza.

In these prior epidemics we found that the subsequent or secondary household attack rates decreased with age. Given an equal exposure, the preschool children had higher attack rates than the school children, which has not been shown (as far as I know) for the Asian influenza. This may be explained on the basis of lack of prior experience with Asian influenza, so that immunity was not noted in the older children.

As reported by Dr. Armstrong for the 1920 outbreak in Kelly's Island and in our studies during

the epidemics of 1952, 1953, and 1955, and also during the Asian influenza outbreaks, it was found that school children were the ones most likely to introduce the infection into the household. With respect to age distribution, in these epidemics the attack rate was also found to be highest among the school children.

With respect to season, the following data might clarify or confuse the picture. We observed two outbreaks of Asian influenza in an institution of about 250 children three to fifteen years of age. The first cases of Asian influenza occurred in early August, 1957, and the outbreak persisted for 37 days, ending about the time school started. There was about 60 per cent turnover of children when the second outbreak of Asian influenza occurred in February, 1958, and lasted only one week. The children lived in seven separate cottages and during the fall outbreaks were frequently mixed together on recreational field trips, et cetera; whereas, during the sharp winter outbreak there was far less intermingling between children of different cottages.

During the fall outbreak, 70 per cent of the children were attacked, confirming a high degree of susceptibility, and yet Asian influenza spread very slowly from one cottage to another. During the sharp winter outbreak only 30 per cent were attacked. Of the children who were not residents in the Junior Village during the first outbreak—we don't know what experience they had had with influenza—39 per cent were attacked during the second outbreak. Only 11 per cent of the children who were in the Junior Village during the first outbreak and also during the second outbreak were attacked. This demonstrates what we think is natural immunity to the Asian strain and which lasted over this short period of time—five to six months. Why the slow spread of the infection in the fall of 1957 and the rapid spread during the winter of 1958? It was not due to the proportion of persons susceptible or to the opportunity for contact spread of infection; it must have been the effect of the season. Season might also account for slow spread of Asian influenza in households observed during the fall outbreak in 1957. I wonder if anyone has data on the speed of household spread during the winter outbreak of Asian influenza.

DR. ROSS L. MCLEAN: Dr. Langmuir's opening remarks concerning the astonishing predictability of the epidemic behavior of influenza points up our complete inability to modify significantly the behavior of such a highly predictable epidemic disease. Although patterns of spread are well documented and transmission may be effected by more than one route, there is but little concrete evidence to say which mechanisms of transmission account for the observed patterns of spread during epidemic and pandemic periods. This discussion will be utilized to present briefly some data which bear upon the question of air-borne transmission of influenza or, in other words, spread by means of infected droplet nuclei.

This study was designed[1] to demonstrate whether the disinfection of droplet nuclei would block the transmission of influenza to a susceptible population during an epidemic period. The concept of "aerial isolation," as described by W. F. Wells, holds that the chief mode of transmission of air-borne infection is via droplet nuclei derived from the evaporation of aerosolized secretions from infected persons, and that the transmission of air-borne infection may be blocked by the disinfection of droplet nuclei.[2] Ultraviolet radiation of the upper air of rooms has been shown to be effective in disinfecting droplet nuclei, but relatively ineffective against surface contaminants at lower levels.[3, 4] Thus, if ultraviolet radiation of the upper air of rooms is associated with a significantly lower incidence of influenza during an epidemic period, this effect may be related to the relative importance of air-borne mechanisms in the epidemic transmission of influenza.

At the Veterans Administration Hospital in Livermore, California, an entire hospital building unit, housing approximately 150 tuberculous patients and patients with chronic pulmonary disease, was totally radiated (figure 4). Facilities for the total care of patients were provided to obviate any need for them to venture outside the radiated area. There remained, nonradiated, another 250 pulmonary tuberculosis patients in various stages of disease and treatment. Samples of serum for anti-

[1] The Advisory Committee which outlined the protocol was composed of Dr. Clifford Bachrach, Chief, Research Statistics Division, Veterans Administration, Washington, D. C.; Mr. J. L. Buttolph, Consulting Engineer; Dr. Charles Chapple, Chief, Clinical Studies Division, Research and Education, Veterans Administration, Washington, D. C.; Dr. Ross L. McLean, Director, Professional Services, Veterans Administration Hospital, Baltimore, Maryland; Dr. Richard Riley, Consultant, Johns Hopkins University; and Dr. Joseph Stokes, Consultant, University of Pennsylvania, Philadelphia, Pennsylvania.

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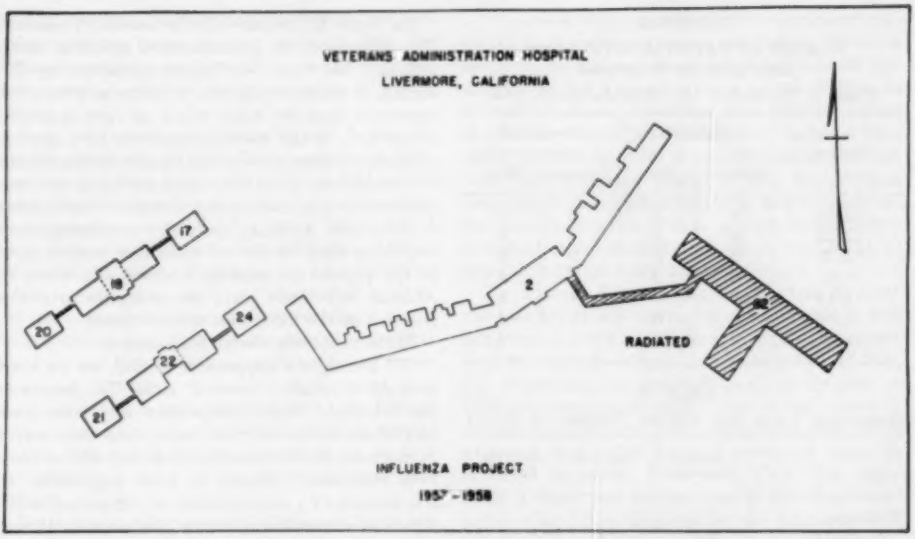

FIG. 4. Illustrates the plan of the hospital grounds and depicts the area which was isolated from the rest of the hospital by radiant disinfection of the upper air of all rooms and corridors.

body studies were obtained from all hospital personnel and all patients at the end of July, 1957, in mid-November, 1957, and again in mid-March, 1958. Virtually none of the patients or personnel were vaccinated.

That portion of the first phase of the 1957–1958 epidemic period in which a sharp outbreak of acute respiratory illness occurred during the second and third weeks of October is outlined in table 8. Results of the mass serologic analysis, carried out at a later date, revealed that this was not Asian-strain influenza, although it was equally apparent that the A2 strain had appeared sporadically among both the radiated and the nonradiated patients. Nor was this one of the other recognized influenza viruses.

In table 9 the events of the second phase of the 1957–1958 epidemic period are depicted. After the October peak subsided, the serologic status of all groups under observation was redetermined by a second mass bleeding in the event that a "second wave" would occur. This "second wave" struck with explosive force during the second and third weeks of January. This was not only a severe, prostrating illness which broke out among the nonradiated patients, but it was unequivocally shown to be Asian-strain disease by subsequent serologic analysis. The radiated patient population was completely spared.

In table 10 all the serologic data for the entire period of the study are summarized without respect to the presence or absence of clinical symptoms. The 2 per cent attack rate for the radiated patients contrasts sharply with the 19 per cent attack rate for the nonradiated patients and the 18 per cent attack rate for the hospital personnel. There would appear to have been virtually complete protection or sparing of the radiated group.

TABLE 8

NUMBER OF PATIENTS WITH ACUTE RESPIRATORY SYMPTOMS

Phase 1, July 28, 1957–November 15, 1957

Week of	Radiated		Nonradiated	
	Influenza	Other	Influenza	Other
9/29	0	0	0	2
10/6	1	3	1	8
10/13	1	1	1	20
10/20	0	1	0	20
10/27	0	5	0	5
11/3	0	0	0	4
11/10	0	0	1	1

In considering the implications of these findings, we must admit that our critics are correct in pointing out that this study lacks the classical controls of identical populations of uniform susceptibility subjected to identical exposure, with a protective influence being applied to one population as the sole variable. A reasonably uniform susceptibility to a new and antigenically distinct strain of virus has been assumed. The fact of exposure has been dem-

TABLE 9

NUMBER OF PATIENTS WITH ACUTE RESPIRATORY SYMPTOMS

Phase 2, November 16, 1957–March 16, 1958

Week of	Radiated		Nonradiated	
	Influenza	Other	Influenza	Other
12/15	0	0	2	0
12/22	0	1	1	5
12/29	0	0	0	8
1/5	0	2	7	4
1/12	0	0	18	6
1/19	0	0	10	4
1/26	0	1	1	1

TABLE 10

SEROLOGIC DATA FOR ENTIRE PERIOD OF STUDY

	Initial Negative	Fourfold Rise	Per Cent Positive
Patients:			
Radiated	209	4	2
Nonradiated	396	75	19
Personnel	511	92	18

onstrated by the appearance of the A2 strain among radiated patients, nonradiated patients, and hospital personnel. Roughly equal exposure of both radiated and nonradiated patients by personnel has been demonstrated, and the over-all attack rate for the nonradiated patients closely approximated that for the hospital personnel.

In addition to these considerations, however, there was a unique form of control in effect—a form of control particularly applicable to the study of air-borne infection—namely, the disinfection of droplet nuclei in the air breathed by the radiated patients. This control was in continuous effect throughout the several months of the study, during which time these patients resided constantly within the radiated area. By contrast, the air breathed by the hospital personnel was not controlled, nor was that of the nonradiated patients.

Hence, only one of the possible routes of infection to the radiated patients was intentionally blocked, namely, that of the infected droplet nucleus, which is air-borne. All other usual routes remained open during the epidemic period of a pandemic due to a new virus. The surrounding community, the hospital personnel, and the nonradiated patients all suffered the usual epidemic experience. The radiated patient group alone remained virtually free of infection, suggesting that an important mechanism of transmission had been significantly blocked during an epidemic period by the radiant disinfection of droplet nuclei.

DR. ALTO E. FELLER (Charlottesville, Virginia): The problem of the mechanisms of spread of Asian influenza has been very lucidly presented by Dr. Jordan. It seems reasonable to accept as a basis for discussion that the Asian strain of virus appeared in one area of the world, presumably by a process of mutation from pre-existing strains. It then spread by established means of communication and apparently by person-to-person contact to many areas of the world. As usual, the really perplexing problems arise when studies are focused on smaller units of the population, such as families, and when, in addition, laboratory data are available to follow the trail of the virus as it spreads about.

Three problems emerge for comment:

(*1*) During the pre-seeding period, do we need to think in terms of "masked" virus? The data from the Cleveland family study show that more than half of the infections with Asian virus were buried in the mass of illnesses classified clinically as common respiratory disease, or were inapparent. In the absence of a large number of influenza-like illnesses to attract our attention, the virus could well be buried in the morass of common respiratory diseases and not masked at all. An important problem for the future is an intensive search for infections in manageable populations during the pre-epidemic period. This will not be easy and will require real dedication.

(*2*) The median interval of six or seven days between the index and all subsequent cases within the family is surprising. It may only mean, however, that we must accept what happens in nature. The concept of "effective exposure" may be a good one even though based largely on ignorance. Perhaps we have relied too much on the results of volunteer experiments which do not necessarily reflect what occurs in nature.

(*3*) Finally, we must return to a study of innate or natural resistance in the host, since nearly half of the persons presumed susceptible did not become infected. Studies of circulating specific antibody have disclosed only part of the answer. Should we not return to studies of nasal secretions, respiratory mucosa, et cetera? In this area there is plenty of room for new ideas.

DR. CLAYTON G. LOOSLI: Dr. C. H. Andrewes and his associates have concerned themselves for many years with problems of epidemic influenza. I am going to ask him if he will please come forward and give us briefly his views concerning the mechanism of spread of this disease. Dr. Andrewes has had no previous warning of this request.

DR. C. H. ANDREWES (London, England): Since Dr. Loosli, as he confesses, has rather sprung this on me, I have only two things I want to say. First of all, I think that the evidence that has been presented today supports what we have gathered

from all sources. This is that the experience with Asian influenza suggests very strongly that the epidemic showed a mixture of two things: a spread from person to person, plus an activation of seeded virus.

Now, as regards the direct spread, I am not terribly impressed by the droplet nuclei. I have been wanting for a long while to try to find a good experimental model for studying the epidemiology of respiratory infections. For some time past, I have been studying with my colleague, Allison, Newcastle disease in day-old chicks. This particular strain is very lethal and very contagious and, in the circumstances in which we are working, it seems to produce a very definite respiratory infection. We find that Newcastle disease spreads very freely across or through a wire grid. If, however, you interpose between your infected and your normal chicks a solid barrier even five inches high, the infection does not get across, suggesting to us that the infection is due to rather coarse air-borne particles.

Furthermore when, instead of normal chicks, we place Petri dishes to collect virus on the other side of the grid from the infected chicks—we put antimicrobials and things in these—we get an abundance of new virus in spite of the fact that a little sawdust is scattered into the dishes. We found that a few inches make a tremendous difference in the amount of virus which would settle out. Therefore, the fact remains that, in the spread of the actual infection which goes all the way around the world in no time, the question of a few inches may make a tremendous amount of difference.

Dr. Jonas E. Salk(Pittsburgh, Pa.): Dr. Feller raised a question concerning the searches for epidemic viruses in inter-epidemic periods. I would like to say that in 1948 and 1949 we were somewhat rewarded in a search for such viruses in patients with pneumonia in spite of the fact that influenza was not occurring in the general population, as indicated by a lack of rise in the incidence of respiratory disease. Patients with bacterial pneumonias not infrequently provided us with strains of virus of antigenic composition similar to those that occurred in the previous influenza season.

I suggest this as a possible source of material. We wondered whether the coincidence of influenza virus and bacterial pneumonia was not the result of activation of influenza virus rather than of the bacterial pneumonia being a complication of the virus infection. The occurrence of antigenic variation resulting from mutation is, of course, an attractive hypothesis. At the time of the study to which I just referred, the possibility was considered but never proved that variants exist in a given population of influenza virus which occurs in the pharynx of an infected individual.

As a result of the appearance of neutralizing antibodies to the major antigenic component, the virus would be neutralized except for those variants that might possibly then exist for a period of time in a "masked phase." I use this term with quotations in that the provocative influence of either a bacterial infection or some of the other influences that have been suggested might, in part, help explain the pattern of antigenic variation and provocation and also the possibility of simultaneous occurrence of influenza in different parts of the world due to somewhat similar antigenic varieties.

Dr. Thomas Francis, Jr.: I would like to point out that Dr. Salk's experience of being able to find influenza in cases of pneumonia in inter-epidemic periods has not been a uniform one. In fact, in 1940, Dr. Brightman, on the third medical division at Bellevue, tried for an entire year to find strains of viruses in patients with pneumonia who were being admitted, and failed till mid-December when an epidemic began in New York City. The same thing, I think, was largely true in Dr. Ward's experience at Johns Hopkins on the failure to find virus in the periods when influenza virus was not present; at least, he failed to find virus in pneumonia patients. I believe Stuart-Harris had certain observations that tended to agree with us, so that Dr. Salk's experience has not been a general one.

Dr. Loosli: We were evaluating an adenovirus vaccine at the San Diego Naval Base when Asian influenza struck the recruit population. It spread most rapidly through the group. Within a period of three days the daily admissions from a population of 4,400 went from 35 to 220. Within three weeks approximately 80 per cent of the recruit population became infected. With the introduction of new recruits the admission rate for Asian influenza remained elevated through the summer and fall months until the first of December.

Dr. Benjamin F. Gundelfinger (Washington, D. C.): We observed at Great Lakes—the Naval Training Center at Great Lakes—a somewhat different behavior of Asian influenza from that Dr. Loosli described at San Diego. We were able to isolate the Asian virus in early August, 1957, and were able to continue isolating it at regular intervals thereafter. However, it was not until early October that the disease became epidemic, manifestated by a sharp increase in admission rates similar to what Dr. Loosli just showed.

During these several summer months the population was continuously changing with respect to composition. The seventeen- to twenty-year-old male recruits were living under the same crowded conditions in barracks and classrooms, working together on the drill fields, and using swimming pools. As far as we could tell there was no difference in their environment and their activities be-

tween the summer months of August and the fall of October. Nor was there any change in the susceptibility of the population as measured by circulating antibody patterns on arrival into the population. However, during the epidemic period of October, the recruits were affected regardless of the length of time after entry into the population.

Influenza is believed to be a highly communicable disease with a short incubation period. Populations whose members live in close contact with each other are usually considered to present an ideal environment for epidemic influenza. The apparent failure of the disease to become epidemic shortly after its introduction into a susceptible crowded population suggests the existence of other factors affecting survival of the virus, its transmissibility, or host susceptibility.

Dr. Joseph W. Cooch (Washington, D. C.): With respect to our experience with Asian influenza at Fort Dix, particular emphasis was placed on the company as an epidemiologic unit. During the extensive outbreak of respiratory disease at this Army post, a large proportion of which was demonstrated to be caused by influenza A (Asian strain), epidemics occurred predominantly within training companies. It is believed that close association within companies favors the spread of the disease. When influenza was less extensive, epidemics of other respiratory disease became more apparent. Epidemics in cadre and support companies did not tend to aggregate sufficiently to become apparent as epidemics. Within any company, attack rates ranged from 5.1 to 35.9 per cent. The intense period of any one company epidemic lasted from one to six days, except in outbreaks with more than one mode; then the entire epidemic within any company lasted from five to forty-two days.

ANIMAL INFLUENZA

JAMES H. STEELE[1]

Equine Influenza

The term influenza has been used to describe acute respiratory diseases in animals almost as long as the word has been descriptive of human illness. Fleming and associates (1), in a history of animal plagues, cites a horse "epizooty," which was described by Arab veterinarians in 1328 in Yemen as possibly being the protean malady "influenza." The first mention of equine influenza in western literature appears in England and Ireland in 1688 (2). The disease is said to have begun and ended its course in a few months. Its onset was sudden, followed by a nasal discharge and collapse. Thousands of horses died. During the pandemic of influenza between 1728–1733, there were many reports of coincident disease in horses and other animals (3). Extensive horse epizootics that were described as analogous to influenza and catarrhal fever were reported in Denmark, England, and Ireland in 1750 (4), and periodically thereafter. In 1781 Gluge (5) tells of a great "epidemy of influenza" which commenced in China in September, 1780, and traveled through Asia, reaching Moscow in December, 1781, spreading across Europe in 1782, and reaching America that spring. Abilgaard (6), the founder of the Danish Veterinary School, and others reported on the concurrent epizootic of influenza in horses. About this time Darwin (7) described an influenza-like disease in horses and dogs.

The nineteenth century medical and veterinary literature is filled with reports of horse influenza epizootics. During the 1803 influenza epidemic, cats, dogs, and cattle were reported to be affected by similar distempers. The 1805 horse influenza was extensively described by many veterinary writers and, from this date on, horse influenza became a specific diagnosis in the European veterinary schools.

Horse influenza epizootics were frequently reported in the United States during the nineteenth century. The last epizootic of any size was that of the winter of 1872–1873 when traffic in New York, Washington, and other cities nearly came to a halt because there were no healthy animals to draw the horse cars, drays, and delivery wagons. As the horse population has fallen from a high of almost 20,000,000 in 1910 to 3,000,000 in 1960, the disease called horse influenza has gradually disappeared.

Jones and associates (8) demonstrated a virus that was thought to be the cause of equine influenza. This agent was not related to the human influenza virus groups. Later European and American investigators identified this virus as being identical with that causing equine virus abortion. Doll (9) believes that equine abortion disease is a sporadic manifestation of the respiratory infection which he calls viral rhinopneumonitis. In 1956, Doll and others (10) reported the finding of another virus, not related to the abortion virus, which produced a respiratory disease and sometimes abortion, and was characterized by plaguelike lesions in the small arteries. It is now thought that this disease probably is the prototype of the old influenza. This disease is called equine infectious arteritis.

The disease only occurs among Equidae. Doll (11) has not been able to produce it in any animals except those of the horse family. It does not grow in the embryonated egg. No vaccines have been developed. Immunity is variable. Older animals that have had the disease earlier in life usually escape later infection, although in some instances they may have a second infection.

The infectious arteritis is highly contagious; practically every young horse, as well as many older ones, will come down with the disease when it appears on the premises. Transmission is presumed to be by droplet infection. The mortality rate is low. The chief loss is from the inability of the animals to work or exercise for weeks or even months.

The onset is sudden, with a high temperature, 103–106°F., which lasts about three days. The animal has no appetite. It is mentally depressed, standing with head down and ears drawn, with no interest in its surroundings or attendants.

Lacrimation and photophobia are usual signs, and often the congested conjunctivae protrude from between the closed eyelids. A mucopurulent discharge from the eyes appears, the corneas be-

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come clouded, and sometimes vision is lost. Nasal discharge is a common sign, and the lymph nodes of the head are swollen. Sometimes there is an edematous swelling of the legs involving the tendon sheaths. Occasionally enteritis occurs. In severe cases, pneumonia is seen which may be fatal.

In 1956 a new type of equine influenza was reported by Swedish investigators that is quite similar to the disease detailed above. The disease was first seen in southern Sweden in 1955. In 1956 the infection appeared in horses at the Dresden spring racing meet. After the meet closed the participating horses moved on to other tracks or returned to their home premises. By midsummer a malady called horse influenza was being reported in eastern Europe. Czechoslovakia had a large epizootic which was studied by both veterinary and medical investigators. Within a short time an etiologic agent, a new myxovirus, was isolated and described by Tumova (12) as being similar to the type A influenza viruses. This agent has been studied by the World Health Organization Influenza Centers and other investigators, and they confirm that the agent is a group A influenza virus. It is of interest that no human disease or infection has been reported among workers handling diseased horses or the virus in the laboratory.

Following the appearance of Asian influenza the World Health Organization requested member countries to survey possible animal hosts, especially horses and swine. Data were collected on antibody levels and influenza A2 isolations from twenty-five countries (13). In the United States no significant A2 antibody levels were found in horses or swine, nor were there any reported isolations of A2 virus (14). Dr. Kaplan of the World Health Organization will discuss the world survey and its implications.

Swine Influenza

Swine influenza is an acute respiratory disease which is frequently seen in the fall and winter. The onset is sudden, and practically all animals in a herd in which the disease appears show signs. Virus pig pneumonia (VPP) is very similar and cannot be differentiated clinically. Another similar disease is forkel grippe, a disease of baby pigs which may cause mortality of 50 per cent or more. This disease has been reported only in Europe.

Swine influenza was first recognized as a new type of disease in the fall of 1918 in Iowa when the pandemic of human influenza was rampant (15). The similarity of the swine disease to that in man probably accounts for calling it influenza or "hog flu." Previous to the 1918 outbreak of swine influenza, there are no reports in the literature of a similar disease in swine that would lead to the suspicion that the infection had existed previously. Most veterinary investigators are of the opinion that swine influenza was contracted originally from man, thus giving rise to a new disease among swine.

Swine influenza for many years was found only in North America. Recently there have been reports that indicate it is now present in swine in Europe (13). There is also a report that the virus has been isolated from rats in Czechoslovakia. The disease in the United States is usually found in the north central states, where it appears annually. The first cases are usually seen in late August or early September after a chilly evening. Shope tells the story that, whenever he was looking for a disease outbreak, he would go to a state fair late in the summer, and usually some swine would come down with the infection and a fresh isolate could be obtained. As early as 1921, veterinary medical observers were finding that large outbreaks of swine influenza were associated with fairs and shows. People who have worked with the disease in the Midwest usually can isolate the virus whenever they seek it during the winter months.

The disease usually appears suddenly in swine herds, and nearly all of the animals develop signs at the same time. The appearance of the disease symptoms in so many animals almost simultaneously has commonly been explained by the extreme contagiousness, but Shope has put forth the conception that the infection is latent and becomes widely spread in a latent form. Then a precipitating agent such as cold weather is responsible for the simultaneous appearance of disease throughout a herd.

The disease begins with fever, anorexia, extreme weakness, and prostration. The animals crowd together, even piling on each other. It is nearly impossible to move them. When moved or handled they are in pain and walk stiffly. A common sign is a jerky type of respiration caused by spasms of the diaphragm which is referred to as thumps. Coughing, indicating bronchitis, is another frequent sign. Some animals develop bronchopneumonia and pulmonary edema. Some of these die. Mortality seldom exceeds 2 to 4 per cent if the

animals are kept warm and dry; otherwise, it may rise to 10 per cent.

On autopsy, the animals show no significant lesions outside the lungs which are characteristic. The involvement is usually bilateral. The diseased area is atelectatic due to a thick mucilaginous exudate in the bronchioles and bronchi. The remainder of the lung is pale because of interstitial emphysema. The cervical, bronchial, and mediastinal lymph nodes are swollen and filled with fluid. When pneumonia occurs, the consolidated portions are atelectatic. The nonpneumonic lung portions in these cases are congested and edematous.

Bacteriologic examination of the disease areas will often yield *Hemophilus suis*. This hemophilic bacillus is frequently found in swine but apparently does not produce disease except when the influenza virus is present. Lewis and Shope (16), when they administered the virus alone to susceptible swine, found that a very mild, almost symptomless, disease resulted. Likewise *H. suis* was virtually nonpathogenetic for swine. However, when both agents were given together, typical influenza resulted. The virus is readily recovered from the nasal secretions, the trachea and bronchial exudate, the lungs, and the lymph nodes draining the lungs.

The question of how the virus exists between seasons has been answered by Shope in an interesting manner (17). He has demonstrated that the swine lungworm and earthworm can harbor the virus for long periods of time. Lungworms, living in the bronchi of affected pigs, ingest virus and the virus is carried through the egg and into the larvae of the parasite. The eggs pass out into the air passages of the pig, are coughed up and swallowed, eventually reaching the soil of the feces. Here they are ingested by earthworms in which the larvae develop, most of them being found in the heart and calcified lymph nodes. They may remain in the worms from one season to another. When the earthworms are ingested by swine, the lungworm larvae leave the earthworm and complete their cycle. Shope demonstrated that when earthworms are fed to swine no infection ensues; but, if the pigs are given several injections of *H. suis*, about one-half will come down with typical swine influenza, and the virus and *H. suis* can be found in the lungs. Shope regards the injection of *H. suis* as a provocation, since he was able to do the same with injections of calcium chloride into the pleural cavity. He has repeated the experiments successfully many times in the late fall, winter, and early spring, but never in the summer. He speaks of the virus in the earthworms as being in a "masked" form. Recently, other investigators have repeated this work in Wisconsin and Nebraska.

The immunity in swine influenza is short. This is evidenced by the fact that swine may often contract the disease several times during their comparatively short life. Neutralizing antibodies are present in the blood for several weeks and the animals are resistant to infection during this time.

The virus is the only part of the two components required to produce infection that will immunize. Shope (18) has shown that the virus confers almost solid immunity to the complex of virus and *H. suis*, while the *H. suis* vaccine will modify the course of the disease but will not prevent it (19). Swine that have recovered from a challenge of a mixture of human ifluenza A virus and *H. suis* are immune to swine influenza virus. Pigs that have received human influenza A virus alone are not immune to swine influenza. Hogs that have recovered from swine influenza are refractory to the human influenza A virus (PR8) (19).

The possibility that human strains of influenza may cause overt disease in swine has been looked into by Shope and others. Shope (19) found in the winter of 1936–1937 that swine on state institutional farms in New Jersey acquired influenza virus of the then current human type A at a time when clinical influenza occurred among inmates of the two institutions. He is of the opinion that, if human influenza could transfer to swine in 1936–1937, there is no reason to believe that it could not in 1918.

Young (20) carried out surveys of butchered hogs in Minnesota during the winters of 1949 and 1950. Of 675 specimens of serum examined for hemagglutination inhibition of Shope 15, Weiss, PR8, Lee, and FM viruses, 16 per cent were positive to 515 virus. Some serum specimens were hemagglutination-inhibition positive to the Weiss virus, but did not neutralize it.

During the past decade, Beveridge, Betts, Hjarre, and other veterinary investigators have sought the virus of swine influenza among pigs in Great Britain[9] and Sweden. They did not find the swine "flu" virus, but Betts and Beveridge did find the cause of a widespread pig pneumonia

[9] A virus was isolated from swine at Cambridge in 1941 which is similar to the Shope strain.

(21). This entity, virus pig pneumonia, is found throughout the world. In the United States it can occur at any time of the year, although it is more frequent during the colder months. The lesions are very similar to those of swine influenza and cannot be differentiated except by serologic methods. This disease and swine flu are usually reported as viral pneumonia by the veterinary diagnostic laboratories. Pulmonary lesions of the disease are often seen on postmortem examination of butchered hogs.

Duck Influenza or Sinusitis

In the spring of 1956, a group A influenza virus was reported isolated from ducks in Czechoslovakia (22). Some 1,250 of 3,000 ducks on the farm were affected with an acute respiratory infection. An acute inflammation of the sinuses was the chief feature. The disease was usually fatal in three to four days. The virus was serologically unrelated to fowl plague (A-fowl) or Newcastle disease virus. In 1959 and 1960 large outbreaks have been reported in other eastern countries as well as in Czechoslovakia.

British Duck Disease

Another virus was isolated from ducklings in England suffering from sinusitis (22). It shows no relation to other myxovirus in hemagglutination-inhibition tests. This disease has not caused any extensive outbreaks such as the Czechoslovakian duck disease.

Fowl Plague

Fowl plague, or fowl pest, is an acute, highly fatal disease of chickens, turkeys, pheasants, and certain wild birds, first recognized as a separate entity about 1880. Ducks, geese, and other waterfowl are less susceptible. Pigeons are resistant. It is caused by a virus that occurs in the blood and affects all organs.

The signs are high temperature, no appetite, and lethargy. The comb and wattles become bluish black. A mucoid nasal discharge appears and often there is edema of the head and neck. The course is rapid and fatal. The mortality rate is close to 100 per cent, which makes it somewhat self-limiting as there are no carriers left. A less deadly form of the disease has been described in Egypt.

The filtrable agent was first demonstrated in 1902. The virus is of interest to influenza workers since Schafer (23) reported that the agent resembles the human influenza A group and that the g-antigens of both viruses are very similar. However, no serologic relations exist between the hemagglutinins of these viruses. Andrewes believes the fowl plague virus has the same right to be considered a species as the swine and horse influenza viruses.

Feline Influenza

This is a common respiratory disease of cats caused by a virus of the psittacosis-lymphogranuloma group. The disease is better known as feline pneumonitis or distemper.

Dog Influenza

There are no valid reports of the recovery of influenza virus from dogs. During the 1957–1958 influenza outbreaks, such reports appeared both in Europe and North America. One such report was received from the Arizona State Health Department, but no further evidence appeared.

World Health Organization Survey

The World Health Organization Serological Survey in animals for type A influenza (13) revealed swine A antibodies in central Europe, e.g., Czechoslovakia and West Germany. Previously a number of European investigators had sought the virus or antibodies in swine populations with no success. Swine A virus or antibodies have not been identified in any other countries during the survey except those mentioned above and in the United States, where Schaeffer and Robinson (14) reported that in 137 cases swine sera showed significant titers against swine/1976/31 antigen. All isolations of influenza virus in swine in the United States since the original 1931 isolation have been of the swine A type.

During the survey no A2 antibodies or viruses were found in swine although they were identified in 288 horses tested. It is also noteworthy that no swine A antibodies were recognized in horses tested.

Concurrently with these studies, swine were infected with A2 virus. These exposed animals did not develop any specific signs of disease, although there was evidence of virus multiplication. Hemagglutination-inhibiting antibody developed in all six swine inoculated intranasally, but complement-fixing antibody developed in none (24).

The antibodies found in the horses were difficult to interpret due to the low titers. In many instances there were no changes between the first and second bleeding, while in others the second serum specimen was found to be negative. Many serum specimens were negative which were collected from areas where an influenza epidemic was experienced. It would seem that the low titers obtained were due to nonspecific inhibition rather than to inhibition by specific antibody.

Following the identification of A equine virus in Prague in 1956, Doll and his colleagues did a survey in Kentucky which is summarized in his report on influenza in horses. It is interesting that Doll and his colleagues have not been able to reproduce the same disease reported in Europe. Apparently, American horses have a much greater degree of resistance to A equine virus.

Bovine Influenza

As with many animal diseases, the name they carry is related to the human disease only because of clinical signs that simulate a like entity in man. Bovine influenza, an example of such an infection, is a disease of cattle characterized by respiratory congestion, conjunctivitis, gastroenteritis, and joint inflammation, which Katsushima first described in Japan in 1889 and 1903 (25), and which was again described in 1916 and 1919 by other Japanese veterinarians. About the same time, bovine influenza was also described in central Europe. For the next thirty years the etiology of this disease continued to be an enigma. The solution began in Iowa in 1940 when McNutt (26) reported on a newly recognized bovine disease which he called sporadic bovine encephalomyelitis. Thirteen years later, Menges and associates (27) and Wenner and others (28) reported that the etiology of sporadic bovine encephalomyelitis was a virus that is antigenically related to the psittacosis-lymphogranuloma group. Shortly thereafter a group of Japanese investigators reported that the bovine ifluenza syndrome was caused by a virus of the psittacosis-lymphogranuloma group (29).

Bovine fever, as it is now called, does not appear to be a single virus or group of viruses, but is caused by a number of different viruses. Since the original isolation, the virus of ephemeral fever, a transitory respiratory fever first described in northern Australia, has been demonstrated in the Japanese syndrome. In 1958 a para-influenza virus that is different from type 3 was isolated from cattle with rhinitis. The following year, a fourth virus that is unlike those of the para-influenza group was recovered from the nasal discharges of cattle suffering from bovine fever.

Bovine respiratory infections are a common problem throughout the world. They frequently are referred to as shipping fever. Shipping fevers are to cattle what the common cold or acute respiratory diseases are to man. Recently, Reisinger (30) has identified a para-influenza 3 virus, SF4, as one of the etiologic agents. Abinanti (31) has reported on a closely related virus as the cause of a crouplike illness in children. These agents are not thought to produce cross infections or pass back and forth between man and animals.

Summary

In summary, it appears that there is evidence to support the belief that some animals are naturally susceptible to human A influenza virus, viz., swine. The observations of Shope and other investigators support this. The more recent findings of the laboratories collaborating in the World Health Organization animal influenza survey indicate that A2 virus will infect horses and swine naturally and artificially. The A2 virus will multiply in swine and is readily recovered.

The two newly discovered strains of influenza A virus, A-equi-Prague-56 and A-duck-57-Czechoslovakia, appear to be widely distributed in Europe. The discovery that fowl plague virus is closely related to the influenza A group reveals that this group has had a long relationship with animals. It is interesting that none of these agents has produced an infection in man and barely affects ferrets or mice.

The possibility of an animal reservoir of influenza A existing in nature must be considered in the light of these new isolations as well as the earlier work. What will happen to these strains in future decades cannot be answered at this time. Surely it should be a very productive area for those who seek further evidence of influenza A in nature.

Shope has asked the question: What will happen with the swine A influenza virus during the next century? Is this agent so well adapted to swine that it will not change or adapt itself to other hosts—or will it eventually die out as animal husbandry methods change—or will it

change and transfer itself to other hosts, including man?

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INFLUENZA IN SWINE AND HORSES[1]

MORRIS SCHAEFFER[2] AND ROSLYN Q. ROBINSON

Serum specimens from swine and horses, collected by veterinarians in different areas of the country, have been examined at this laboratory in conjunction with the World Health Organization survey to determine the role of swine and horses in the epidemiologic cycle of influenza. This survey was initiated after reports from Europe indicated that antibodies against influenza strains, commonly found in humans, had been found to be present in horse serum.

To verify this observation, initial serum specimens were collected from swine and horses prior to an epidemic of human influenza, and second serum specimens were obtained after an epidemic. The paired sera were then used in the hemagglutination-inhibition test for determination of specific antibody titers against the swine/1976/31 and A/Asian/Japan/305/57 influenza strains. All sera were treated with trypsin and potassium periodate for the removal of nonspecific inhibitor. Swine sera containing antibodies against A/Asian/Japan/305/57 through experimental infection were used, together with other control sera, to ensure that a suitable method of testing was being employed.

A total of 844 serum samples was examined by this technique. In table 11 are shown the geographic locations from which the sera were collected together with numbers studied and numbers showing specific antibody titers. Of the 844 serum specimens, 288 were collected from horses.

In no case did horse serum show evidence of the presence of antibodies against the swine/1976/31 antigen. In 51 of the 288 cases, titers were obtained in horse serum against the A/Asian/Japan/305/57 antigen. However, these results are difficult to interpret due to the fact that the titers were very low and sporadic. In many cases in which a low titer was observed, there was no change between the two serum specimens, and low titers were occasionally obtained in the first serum specimen while the second serum specimen was found to be negative. Many serum specimens were negative which were collected from areas where an influenza epidemic had been experienced. It would seem that the low titers obtained were due to nonspecific inhibition rather than to inhibition by specific antibody.

TABLE 11

SUMMARY OF HEMAGGLUTINATION-INHIBITION TEST RESULTS WITH HORSE AND SWINE SERA

Geographic Location	Horse Sera			Swine Sera		
	Total	A/Asian/Japan/305/57 Positive	Swine/1976/31 Positive	Total	A/Asian/Japan/305/57 Positive	Swine/1976/31 Positive
Louisiana	36	11	0	134	0	14
Oregon				10	0	0
Wisconsin	6	0	0	184	0	96
West Virginia	53	4	0	13	0	0
Alabama				154	0	27
New York	29	0	0			
Arizona	86	36	0	108	0	0
South Carolina				1	0	0
Massachusetts	18	0	0	12	0	0
Total	228	51	0	616	0	137

In no case did swine sera show the presence of antibody against the A/Asian/Japan/305/57 antigen. However, in 137 cases, swine sera showed significant titers against the swine/1976/31 antigen. On the basis of these results, it would appear that cases of influenza in swine have been due to the swine set of influenza viruses. This is further supported by the fact that, in October, 1957, Dr. Robert Hanson at the University of Wisconsin made four isolations of influenza virus from swine which were closely related antigenically to the prototype swine isolation in 1931, as have been all influenza virus isolations from swine since that time.

Results of this study indicate that there is good evidence that the Asian set of influenza viruses was not established in the swine population and, although low titers were obtained in some cases in horse sera, the nature of the results do not indicate that this set of influenza viruses became established in horses.

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INFLUENZA OF HORSES[1, 2]

E. R. DOLL

Introduction

The term influenza has been used rather indiscriminately in America for describing respiratory diseases of the horse. A wide variety of symptoms and lesions are found as well as complications described under the name of influenza in veterinary textbooks. A few writers have tried to separate respiratory disorders on the basis of clinical signs, a quite difficult undertaking because symptoms and severity vary greatly according to conditions under which infection occurs.

Two distinct viral entities have been identified in the old equine influenza complex of horses. A new name has been applied to each disease according to its pathologic and clinical features. The two diseases will be considered only briefly because these viruses are not related to the influenza viruses of man and swine. There is no evidence that either virus is infectious for man and, therefore, they do not present a problem in public health. Each disease, however, is unique in some respects and could provide a model for research in comparative medical biology. The two diseases are viral rhinopneumonitis and viral arteritis.

Observations

Viral Rhinopneumonitis

Viral rhinopneumonitis of horses was investigated initially as equine virus abortion in Kentucky and as equine influenza in Virginia and Maryland. The first association of the abortion disease with a respiratory catarrh regarded as influenza was made by investigators in Hungary. Comparative study of the abortion virus from Kentucky and the influenza virus recovered from Army Remount horses in Virginia revealed that the two viruses were identical.

The uncomplicated viral infection causes a fever with temperatures varying from 102–105°F. for periods of one to five days. The fever is accompanied by a leukopenia and a serous respiratory catarrh. Appetite may be impaired and activity reduced. Often the disease is asymptomatic. In farm horses, the complications are seldom more serious than a mucopurulent rhinitis that persists for one to four weeks. Pneumonia may occur in horses subjected to shipping and hard work. The virus has a marked affinity for infecting the fetus when pregnant mares contract the disease. Infection of the fetus appears to be a disease subsequent to, but independent from, that of the mare. Abortions occur from three weeks to three months after infection of the mare. The fetus usually is expelled without premonitory symptoms of impending abortion.

The mare suffers no ill effect, either from the viral infection of the fetus or in future breeding performance. Rhinopneumonitis viral infection of the equine fetus is characterized by pulmonary edema, hydrothorax, and focal necrosis of the liver. Intranuclear inclusion bodies occur in the hepatic cells, in the bronchiolar epithelium, and in the alveolar cells. Ninety-five per cent of abortions occur from the beginning of the eighth month to full-term gestation.

The rhinopneumonitis virus may be isolated from aborted fetuses by intraperitoneal inoculation of suckling hamsters, in which it produces a fatal hepatitis with intranuclear inclusions in the hepatic cells. From hamsters, the virus may be adapted to embryonated chicken eggs, but it grows poorly in this host. It also has been isolated from equine fetal tissue by intracerebral inoculation of suckling mice, but does not propagate prolifically. The virus may be isolated either from nasal washings of horses or from fetal tissues in monolayer cultures of equine kidney. It has been propagated in several tissues in the Maitland type of cultures and in HeLa cells. Complement-fixation antigens may be prepared from lung tissue of equine fetuses or the livers of hamsters. LD_{50} titers of 10^8 to 10^9 per gm. of hamster liver are attainable after the virus is fully adapted. Precise *in vitro* neutralization tests are possible by intraperitoneal inoculation of hamsters.

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Viral Arteritis

The equine arteritis virus was isolated in our laboratories from an aborted fetus in 1953. The fetus was submitted from a farm on which several mares had aborted, and many horses had symptoms compatible with some described for equine influenza. Our knowledge of the disease was derived almost entirely from experimental inoculations. The disease has occurred sporadically in widely separated states during the last six years. It occurs very infrequently compared with rhinopneumonitis.

Approximately 30 per cent of our experimental cases terminated in death. Pathologic studies revealed that the specific lesion of the disease is a degeneration and necrosis of the media of small muscular arteries. The arterial lesions occur in all parts of the body. It appears that other lesions and symptoms are derived from damage to the arteries which results in hemorrhage, thrombosis, infarction, and edema.

The disease is initiated by a fever with temperatures varying from 102–106.5°F. and persisting for three to seven days. Leukopenia parallels the fever, and the lymphocytes are the most severely affected cells of the leukocyte group. Other symptoms are serous nasal efflux, photophobia, conjunctivitis, palpebral edema, loss of appetite, dyspnea, colic, diarrhea, dehydration, icterus, severe depression and weakness, and edema of the legs. Lesions are primarily edematous. Edema occurs in the lungs, the mediastinal tissues, the fat at the base of the heart, the mesentery, the intestines, and the uterus. The pleural and peritoneal cavities may contain from a slight excess to several gallons of the serum-like fluid, which may contain fibrin clots. Enteritis occurs frequently. It varies from a simple catarrhal inflammation in the small intestine to a severe hemorrhagic and diphtheritic condition in the cecum and colon. Hemorrhages occur on all serous surfaces and many mucosal surfaces.

The most severe loss from the disease under farm conditions is from abortion. Seventy per cent or more of pregnant mares may abort. Abortion occurs during the terminal part of the acute phase or early in convalescence. Future breeding performance of the mares appears to be unaffected. The aborted fetuses have no specific lesions, either gross or microscopic. The virus is readily isolated from aborted fetuses, provided that they are expelled before becoming severely autolyzed.

The virus has not been propagated in laboratory animals or chicken embryos. Recently, it has been propagated in monolayer cultures of equine kidney. We have no serologic tests for identifying the virus. Definitive diagnosis is presently dependent upon cross-protection tests in horses. Equine viral arteritis appears to be identical with the epizootic cellulitis-pinkeye syndrome that was described in accounts of equine influenza.

Equine Influenza

The new names, rhinopneumonitis and arteritis, were proposed for the two equine diseases described above because the two viruses, as well as the diseases induced by them, were incompatible with the influenzas of man and swine. The recent isolation of the A-equi-Prague-56 virus in Czechoslovakia from horses during an epizootic of respiratory disease seems to fully justify our decision to cease using the term "influenza" for the diseases just described. Investigators in Czechoslovakia have expressed the opinion that the disease caused by the A-equi virus should be designated as equine influenza. Since the virus appears to be a typical member of the influenza group, this terminology should be acceptable.

We have only very sketchy information on the natural disease as it occurred in Europe. The disease appears to be a febrile respiratory catarrh, with fever varying from 2–4°F. above normal for periods of three to five days. Horses that are sheltered and kept at rest apparently seldom develop complications. Hard work, shipping, exposure, et cetera, apparently may result in the complications of the "shipping fever" disorders, which may also accompany rhinopneumonitis or arteritis.

Our experience with the disease in the United States has been limited to serologic surveys and to attempts to study experimental disease caused by the A-equi virus. The A-equi virus available for experimental studies had been passed five to eight times in chicken embryos. The inoculations were made either intranasally or intratracheally in weanling or yearling horses. The dose of virus varied from 6 to 10×10^7 embryo-infective doses for each horse. We have not produced a clearly discernible disease with the embryo-propagated virus. Of more than 20 experimental subjects, more than one-half did not have fever detectable by rectal temperature readings taken twice daily. Febrile reactions in the remaining horses were questionable, being not

more than 0.5–1.0°F. above the upper normal range. Roughly one-half of the experimental subjects had a slight watery efflux from the nostrils from the second to fourth days after inoculation. Horses inoculated intratracheally developed hemagglutination-inhibition titers varying from 1:20 to 1:80. Those inoculated intranasally developed hemagglutination-inhibition titers varying from 1:5 to 1:20. There was no disturbance of the leukocyte count. The low titers and failure to produce clinical disease suggest loss of virulence during embryo passage of the virus.

Our serologic surveys were conducted on serum specimens obtained from horses employed in vaccination trials with a rhinopneumonitis vaccine.

Only pre-vaccination serum specimens were used in the hemagglutination-inhibition tests for A-equi. All sera were heated to 62°C. for thirty minutes before testing. After finding a number of positive sera, 19 with titers varying from 1:10 to 1:80 were selected, and aliquots of each were subjected to heat at 62°C. for thirty minutes in a water bath, standard trypsinization treatment, and standard periodate treatment.

Comparable titers resulted from each treatment. Another group of 314 horse sera was heated at 62°C. for thirty minutes and employed in hemagglutination-inhibition tests against A swine/1976/31, A (PR8), A1 (FM1), A2/Formosa 313/57, A1/Denver/57, B (Lee), Sendai, Enders mumps, and Dintner N virus. Agglutination was not inhibited completely at the 1:5 dilution by any serum-virus combination. It appears from these results that there is little problem of nonspecific inhibition by horse sera heated at 62°C. for thirty minutes in hemagglutination-inhibition tests with the influenza viruses. These results would seem to lend validity to the findings in hemagglutination-inhibition tests with the A-equi-Prague-56 influenza virus.

Hemagglutination-inhibition tests were made on sera from 198 yearlings in 1957 and 1958, 53 per cent being negative or incomplete at the 1:5 dilution, 35.8 per cent giving complete inhibition at 1:5, 7.5 per cent giving complete inhibition at 1:10, 2 per cent inhibiting completely at 1:20, 0.5 per cent at 1:40, and 1.0 per cent at 1:80. Sera from 792 mares were tested for hemagglutination-inhibition reaction against A-equi in 1957 and 1958, 27.6 per cent being negative or inhibiting incompletely at the 1:5 serum dilution. The percentages of sera giving complete inhibition for the respective dilutions were: 22.6 per cent at 1:5; 23.2 per cent at 1:10; 16.4 per cent at 1:20; 6.5 per cent at 1:40; 3.1 per cent at 1:80; and 0.25 per cent at 1:160.

The titers obtained from sera of horses included in the survey compare favorably with or exceed those obtained from horses inoculated with the embryo-passaged virus. The titers of both groups, however, are considerably lower than those reported by Sovinova and co-workers for convalescent sera from horses in Czechoslovakia. The differences could be due to remote infection of the mares, but any infection of the yearlings would have been relatively recent. This suggests the possibility that titers obtained in our subjects resulted from a related, but not identical, virus. Influenza of horses in the United States, whether caused by A-equi or a related influenza virus, has not been established conclusively by isolation of an influenza virus or by demonstrating a differential in titer of sera from the acute phase and convalescent period.

General Discussion

Dr. M. M. Kaplan (Geneva, Switzerland): Dr. Steele brought up the question of animal influenza, a field which many of us believe has been neglected in the attempt to work out the natural history of influenza in general, especially as it affects human beings.

In 1957 when the Asian pandemic struck, Dr. Payne and I, with a group of influenza specialists who happened to be in Geneva, discussed the advisability of undertaking a study in animals throughout the world in conjunction with this pandemic to determine, or rather to try to unearth, more facts about animal influenza, and its prevalence in different countries, and to see what the action of the A2 virus would be in domestic livestock which we knew were susceptible to influenza strains. This survey was undertaken, as indicated by Dr. Steele, in some twenty-five countries. The results were published in the World Health Organization *Bulletin* last year and I would refer you to that article for details of the countries involved, the tests that were used, and the conclusions that were drawn (Kaplan, M. M., and Payne, A. M.-M., Bull. WHO, 1959, *20*, 465).

The report of the isolation of the A2 virus from pig lung in China, where the pandemic started, prodded us a great deal to undertake this survey. I might say at once that we have no reports other than the China report and one from Spain on the isolation of the virus from natural infections in

animals. The Spanish isolation was from pigs from the abbatoir of Madrid. This strain was sent to London. There was little question that it was an A2 virus and the question, of course, arises whether it was a laboratory contamination or a primary isolation. In trying to determine this through very close questioning, it would appear that it was a natural isolation. The new workers with the A2 virus in the laboratory had not worked with the virus in the laboratory prior to its isolation. There was no reason to suspect any contamination of the primary material. Of course, a slight question about that will always remain in our minds, but this is the information we have obtained. As Dr. Steele indicated, the survey we conducted showed that influenza A2 virus can cause natural infection in both horses and swine. This is not surprising; I think of Shope's work back in the thirties in which he showed that the swine population in the human epidemic area reflected or indicated naturally acquired infection.

This A2 finding is merely an indication that this does happen and can happen, although we haven't yet been able to observe any clinical reactions worth speaking of in artificially infected swine or horses or in natural infections that were determined by a serologic survey. Thus we do not have any information of the clinical picture that was evolved.

The other interesting thing that was uncovered by serologic analysis was the world-wide prevalence of the equine influenza virus. Again I must stress that a serologic survey, as all of you know, has its great pitfalls, such as the question of the tests, the uniformity of the reagents involved, the question of nonspecific inhibitors, et cetera. Taking into consideration all of these factors, we tried to analyze in our paper—and our conclusions are based on the elimination of anything that would becloud the specificity of the tests—the height of the titers and the treatment of the serum for the non-specific inhibitors. I think that the occurrence of natural infection can be accepted.

The equine influenza virus, besides having been present in Sweden where it was first reported in 1955, produced a widespread epizootic in Czechoslovakia. It was also identified in such countries as Rumania, and by serologic procedures in the United States, as a rather widespread infection due either to the A-equi-Prague or a very closely related strain. Antibodies due to this strain were also apparently identified in Denmark, Finland, the Federal Republic of Germany, Japan, the Netherlands, Poland, the Soviet Union, and also perhaps in Australia. However, this only indicates that we have a great deal to learn about animal influenzas and their prevalence. We hope we have done one thing in the survey—awakened interest in this field of influenza investigation so that it can be followed with greater detail and depth in the various countries of the world.

These findings bring up certain epidemiologic considerations on the question of animal influenzas in general. One is the very interesting theory that Dr. Mulder has propounded. He postulates that perhaps the major pandemics, of which we have a fairly good record beginning in the last century, arose from the same area in China or the same general area in China, and the possibility presents itself that we are dealing with an animal reservoir as the origin of the pandemic. We have tried to encourage or tried to get studies under way in China concerning wild and domestic animal fauna but we have not met with any success until now. Unfortunately, mainland China is not a member of the World Health Organization, and nothing could be done in that line. However, there is some work, I understand, under way there by the Chinese authorities and the scientists themselves. One of them, Dr. Chu, has been trained in England in influenza work and has done very reliable studies. We hope that from their publications we will learn more about the fauna and what is happening in that particular area.

The other aspect that I think is worth touching on is the question of swine influenza and the natural history of the disease. We have a much greater opportunity in the animal populations to get some leads in what happened from an evolutionary point of view or in the natural history, let us say, of influenza. One hypothesis of the 1918 pandemic as affecting swine for the first time is a very interesting one, but I must confess that I find it a bit difficult to accept, primarily from the following point of view. We are presented with a complex biologic cycle involving a lungworm, an earthworm, and an animal host (a pig). From deduced knowledge of the natural history of disease, it would take eons of time—let us say at least a long period of time—for this very close adaptation to occur leading to this complex biologic cycle. Now what are we asked to accept here? (I am not saying that Shope claimed this specifically, but his data must be accepted as fact.) If, as Dr. Steele indicated, there did not seem to be a disease of pigs, a swine influenza, before the 1918 epidemic so far as we can gather from our records, what we are accepting is that a phenomenon normally requiring a long period of biologic adaptation happened once and only in the United States. This would require a very remarkable set of circumstances, I think, to have occurred at that one particular place and period and nowhere else.

Many remarkable things have happened in the biologic field in the course of evolution. I think, however, that this interpretation of Shope's data

stretches one's imagination a little further than is ordinarily expected. But there must be some explanation. Whether the above is the right one, I can't say. None of us can say. It does point up the necessity of investigating the animal influenzas in all their aspects. The World Health Organization is planning to do so further in a serologic survey of animal influenzas using the complement-fixation tests of Henle and Lief as the epidemiologic tools. It plans to extend its investigations on a worldwide scale to include wild animal reservoirs as well as domestic ones in primitive communities and advanced communities, in relation to pandemic and interepidemic periods. Perhaps we can shed a little more light on this very fascinating and important aspect of influenza.

DR. C. A. BRANDLY (Urbana, Illinois): Much of our present image of the epidemiology of the influenzas is the product of retrospection—and retroaction. Partly through advances in methods and in part as a consequence of earlier lack of foresight, we have repeatedly gone back to "rummage" in the attic of indirect evidence. The "butter mold or imprint" implement has received the most intense scrutiny, and this effort has yielded facts to greatly fortify our hindsight. Therewith, of course, our perspectives have been magnified to a remarkable extent.

The excellent reviews of Drs. Jordan and Steele together with Dr. Kaplan's comments and the documented data and evaluations of many others from our own and other lands provide us with an inviting and challenging basis for critical and fruitful future effort.

Swine influenza prompted Shope's brilliantly stimulating and productive work. The end product has been a far more imaginative approach to the inter-epidemic whereabouts of this and other viruses, i.e., that they may reside within various orders of animal hosts, and the wide acceptance that man was the original donor of the virus to swine. And it has better oriented the concept of stress (microbic, meteorologic, and other) in precipitating infectious disease. Certainly, the impact and implications of Shope's (1931) "filtrate disease" on a single phase of influenza epidemiology can scarcely be overestimated.

Now, with nearly three decades of identified or recognized influenza behavior "under our perspective belts," it is suggested that the prototype of influenza, in both man and others of the animal kingdom, may have a lair in animals on the Chinese mainland.

Perhaps a few questions may be warranted, in view of our expanding realizations. One arises from a somewhat *restricted* veterinary medical approach. It is based on the responsibility of protecting our food-producing animals. With benefit of advanced, precise diagnostic techniques, and the advantage of methods for stamping out animal disease together with enforceable quarantine and regulation procedures, could we eradicate swine influenza? If we answer affirmatively, more questions will be forthcoming. I will answer that it should indeed be possible! We have twice proved this capability in this country—in 1925 and 1929—in dealing *epidemiologically* with a possibly less agile and versatile agent of the influenza group. The relative newcomer to the group to which I refer is, of course, classical fowl pest or plague (not its Egyptian variant). Yet, the problem of breaking the cycle and eradicating such a malady of domestic animals is basically not an epidemiologic one (or is it?). Rather it is one of establishing an *adequate* public conscience and of the necessary mobilizable finances to accomplish summary stamping out of the immediately recognizable reservoirs in infected flocks and individals, plus, of course, the applicability of current knowledge of how to destroy the occult infectious agent as well as its overt, exposed form. Again, there is an irrational obstacle frustrating the veterinarian in his approach. One example is that of African swine fever, possibly, but probably not, a myxovirus of the influenza family which has frightening possibilities. (It is a fulminating and fatal disease adapted to wart hogs and largely unrestricted by natural immunologic capacity of the host swine species of other areas.) To guard against introducing this disease, and also foot and mouth disease, the Animal Quarantine and Inspection Division of the federal Agricultural Research Service has barred further imports of wart hogs and other feral African swine, and also of some ruminants. Even with the threat of eventually restricting or denying themselves steaks and pork chops, a group of zoo directors (in 1958) strongly opposed this action on restricting such imports. This, because it kept them, temporarily at least, from replenishing or adding to their zoos.

Kaplan and Payne suggest that imported American pork products are responsible for serologic evidence of swine A influenza in West Germany and Czechoslovakia. Why, however, didn't the disease spread to Australia by the same means? Koen's observation in 1918 that swine and human influenza outbreaks were related and due to the same contagion touched off real economic concern lest pork be barred from the tables of many.

While this and similar matters may seem remote to the main problem of influenzal epidemiology, they are a part of the over-all picture and cannot be disregarded. Perhaps it's easiest to dismiss them, however, as problems of future educational enlightenment.

The intensive exploration of the entire fauna of

a region, as has been suggested, in order to ascertain the host spectrum or reservoir of influenza viruses is at the outset an exciting yet colossal undertaking. Nevertheless, it may eventually become an epidemiologic necessity. There is no longer a question but that others of the animal kingdom, as has been re-emphasized, are components of the influenzal disease entity. Candor requires that the task not be undertaken in a piecemeal manner but that long-range planning be initiated and that a full complement of workers from each of the cognate biologic disciplines be set at the task. In this direction, it seems essential that possible multiple hosts and reservoirs of contagion receive more critical, intensive consideration.

Finally, it may be pointed out that modern improved environmental sanitation, as it can increase the production and well-being of animals, is often largely neutralized by the hazards of their further crowding under highly artificial circumstances. With consequent increased contacts among the animals, the respiratory contagions, especially, are unduly favored both in perpetuating themselves in one or more animal populations and in their potential for spread and adaptation to man and other species. Nevertheless, with rearing and maintenance of animals within a narrower area of residence, we may expect progress toward defining and imposing the measures necessary to suppress the spread of various contagious diseases.

Dr. R. P. Hanson (Madison, Wisconsin): Dr. Ewing, a pathologist on the swine influenza project in our laboratory at the University of Wisconsin, observed that in nature it was very rare to find a pig infected with only a single species of swine lung worm. Almost all individuals were infected with two or three of the three species of swine lung worms found in the United States. He attempted infections with single species using 50 to 500 third-stage larvae. He was unable to produce infections. If he used the same total number of larvae of two species, 50 per cent matured and produced eggs. If he used massive infections, 6,000 or more larvae of a single species, he occasionally obtained an infection. In these instances, less than one per cent of the lung worms developed to maturity.

Dr. Fukumi: I would like to add one fact to Dr. Steele's speech. I do not know if it is correct or not to call it swine para-influenza or something like that, but at any rate there is a mild febrile illness of pigs in Japan caused by a so-called Sendai virus, a member of the myxovirus group. This virus also was and still is found to cause a latent infection in epidemic form among mouse flocks in Japan from time to time, and thus presents some difficulties in virus isolation experiments by using intranasal inoculation into mice. It was occasionally isolated also from guinea pigs showing a pneumonic affliction. So far no connection has been found between the Sendai virus infections in swine and the laboratory animals. Furthermore, no difference has been found either serologically or biologically between Sendai virus strains isolated from swine and mice. It is not certain whether there are mouse-proper and swine-proper Sendai viruses, or whether there may be some common virus reservoirs or vehicles which cause infections either in swine or in mice. Para-influenza virus type 1, which is isolated from human para-influenza cases, is clearly distinguished from Sendai virus serologically and biologically and is not pathogenic for mice or guinea pigs, while there is no evidence, at least in Japan, that Sendai virus causes a disease in human beings. Thus, there is no need to worry about the possibility that Sendai virus infections in swine or mice may become virus reservoirs for a human infection.

Dr. G. O. Broun (St. Louis, Missouri): I understand the great problem of the nonspecific inhibitors of the antihemagglutinin reaction and realize also that the finding of an antibody does not mean the presence of a virus. We ran a very small number of sheep, cattle, and chicken sera, collected before, some during, and some after the 1957 epidemic, for antibodies to the A2 virus. In the specimens obtained before and during the epidemic, we found no antibodies. Beginning with specimens obtained during May, 1958, we found a few with low titer antibodies against A2 virus. Admittedly, the strains of virus that we were using are highly sensitive to nonspecific inhibitors. We did treat the serum with periodate according to the method recommended by Jensen in 1957.

Dr. K. E. Jensen (Terre Haute, Indiana): I had hoped that Dr. Fred Davenport would make this comment because I know that he and Dr. Peterson have continued some studies that were begun some years ago at the University of Michigan about the nature of swine influenza viruses. The thing that we were struck with at that time was the amazing antigenic constancy of the isolates that had been obtained from 1931 through 1958. The antigenic relationship stayed very much the same. I would like to suggest to Dr. Kaplan that the reason we have this swine influenza cycle is that this is a chance phenomena. This particular virus probably does have the capacity to maintain itself in this cycle, where perhaps another virus would not.

CLINICAL CHARACTERISTICS

ALTO E. FELLER, *Chairman*

TWENTY YEARS OF INFLUENZA EPIDEMICS

C. H. STUART-HARRIS[1]

Introduction

May I first say what an honor it is to be invited to participate in this International Conference on Asian Influenza. As we already learned from our morning's discussion, it is quite obvious that we are not going to limit ourselves (I hope) just to the consideration of that epidemic. And so I have entitled my own contribution *Twenty Years of Influenza Epidemics*. I will begin with a quotation:

> A kind of fever, in general slight, but some times not a little fatal to old men and children who had weak lungs, raged throughout all this country. At once and at the same time, innumerable persons were seized with a wandering kind of shiver and heaviness in the head; presently also came on a pain therein and also in the joints and back; several however, were troubled with a universal lassitude. Immediately a very great and acrid defluxion from the eyes, nostrils and fauces, and very often falling upon the lungs, which occasion almost perpetual sneezings, and commonly a violent cough.

Asian influenza in 1957 in Ruritania? No, merely a description of an influenza epidemic in 1743 written by a country doctor, John Huxham (1), in Plymouth, England. I have quoted this paragraph fully because it emphasizes the first point which any clinician should make in describing the impact of influenza on his patients. Epidemics come and epidemics go, but the clinical picture of influenza remains remarkably unchanged.

Because a clinician's impressions are, however, notoriously fallible, it is important to examine first of all the evidence that Asian influenza was, in fact, not different from ordinary influenza as we have known it in our medical lifetime. In compiling this evidence, I have drawn extensively from the many excellent and detailed descriptions of Asian influenza which have appeared in the American literature. Moreover, I have been struck by the fact that some of these writers have been at pains to stress the resemblances which they noted between Asian influenza and earlier epidemics since the first recovery of influenza virus in 1933.

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The Uncomplicated Disease

Symptoms

Because influenza is a disease with few characteristic signs, symptoms are particularly important. In table 1 three groups of patients are compared. In the first column are listed symptoms in sixty personally observed and virologically proved cases of influenza A, drawn from the three epidemics of 1937, 1939, and 1941 (2). In the second and third columns the frequency of the same symptoms is shown in twenty-eight proved cases of influenza A1 from 1950 to 1956 and in thirty cases of Asian influenza in 1957 from the Cleveland family studies, as recorded by Jordan and others of Dr. John Dingle's group in 1958 (3). The resemblances between these three groups of patients outweigh the observed differences; and yet it must not be forgotten that, although all three groups of patients were aged fifteen or more, my patients were servicemen eighteen to twenty-five years old and the Cleveland patients were adult civilians living at home. The sudden onset of illness noted in three-fourths of my own patients was apparently less common in Cleveland and yet was particularly noted by other observers as being characteristic of Asian influenza. The fact that constitutional symptoms were present in at least three-fourths of the patients in all three groups emphasizes the ability of influenza to cause general illness. As a medical officer at a school expressed it, "The boys were more generally 'felled' and apathetic than is usual; 'I suddenly began to feel terrible' was frequently heard when taking histories." Dizziness, a curious symptom for a short febrile upset, though not mentioned in the Cleveland patients, was emphasized by many other com-

TABLE 1

SYMPTOMATOLOGY OF INFLUENZA A, A1, AND A2 IN ADULTS (PERCENTAGE FREQUENCY)

	1937, 1939, 1941 Influenza A (60 Cases*)	1950, 1951, 1953, 1956 Influenza A1 (28 Cases†)	1957 Influenza A2 (30 Cases†)
	per cent	*per cent*	*per cent*
Sudden onset	75	32	46
Premonitory	18	—	—
Constitutional symptoms:			
Malaise	87	71	66
Headache	85	82	72
Shivering	80	78	64
Anorexia	71	64	37
Muscular pains	60	78	62
Dizziness	32	—	—
Ocular symptoms	20	—	—
Respiratory symptoms:			
Cough	88	93	90
Coryza or nasal obstruction	80	93	82
Sore throat	48	75	62
Expectoration	31	55	40
Chest pain	11	—	—
Hoarse voice	10	32	37
Miscellaneous:			
Insomnia	38	0	0
Nausea or vomiting	17	3	11
Abdominal pain	0	0	0
Diarrhea	0	11	0
Irritability	0	43	22

* Reported in (2)
† Reported in (3).

mentators on Asian influenza and cannot therefore have been only a feature of 1937 influenza.

The respiratory tract symptoms noted almost uniformly in influenza are cough and either nasal discharge or stuffiness. Yet the cough is more often unproductive than accompanied by sputum, and substernal soreness or pain is relatively infrequent in previous healthy adults. The variation in percentage of those complaining of sore throat or of hoarseness is more difficult to explain. One must remember that observer variation is at least as important as the variation among patients. I suspect that the recording of these symptoms is much influenced by the severity of the complaint. Soreness of the throat in influenza in 1937 was not impressive to me. Yet, during the Asian epidemic many stated it to be almost invariable. Probably the figure of 60 per cent from Cleveland represents the usual experience, and yet it was less frequently recorded then than in the influenza A1 cases. Hoarseness of the voice is also assessed differently by different observers and many patients with a little huskiness were not included in my 1937 series. In table 2, compiled by Lackman and associates (4), the comparability of groups of patients with Asian and A1 influenza is also illustrated. As these groups were derived from the same population—the University of Idaho—there was at least this common factor; and perhaps this accounts for the well-nigh identical percentages for the various symptoms.

All observers are agreed that there is a very low incidence of gastrointestinal symptoms in influenza, and this is doubtless true for adults. But vomiting is much more common in children with influenza than in adults, and it was found in one-fourth of the children observed at Cleveland in 1957 and in the earlier years. At least we are at one in commenting on the absence of diarrhea as a symptom of influenza.

Signs

As with the symptoms, so also the physical signs of influenza show remarkably little variation from one epidemic to the next. The signs observed in four series of patients are assembled in table 3.

The fever of influenza is a characteristic of the disease, yet there are relatively few accurate accounts of it. In 1937 the average duration of

TABLE 2

SYMPTOMS AT ONSET OF INFLUENZA, UNIVERSITY OF IDAHO*

	Influenza A2, October 1957 (602 Cases)	Influenza A1, March 1957 (608 Cases)
	per cent	*per cent*
Sudden onset	More than 50	More than 50
Headache	64	58
Sore throat	61	46
Fever	58	51
Cough	56	48
Chills	46	37
Weakness	46	37
Nasal obstruction	41	36
Muscular pains	40	39
Sweating	35	31
Dizziness	32	26
Anorexia	21	28
Insomnia	20	24
Nausea	15	17

* Reported in (4).

TABLE 3

PERCENTAGE OF ABNORMALITIES IN UNCOMPLICATED INFLUENZA

	1937 Influenza A (England)† *82 Cases*	1943 Influenza A (U.S. Army*) *74 Cases*	1950–56 Influenza A1 (Cleveland) *28 Cases*	1957 Asian influenza (Cleveland) *30 Cases*
Fever:				
Mean duration	3.6 days	—	—	—
Highest temperature (average)	101.2°F.	<100°F. 11	<100°F. 13.1	<100°F. 12.5
		100–101.9°F. 38	101–101.9°F. 47.8	100–101.9°F. 58.3
		102°F. + 50	102°F. + 39.1	102°F. + 29.2
Flushed face	69	45	8.7	24.0
Conjunctival abnormalities	89	46	13.0	56.0
Nasal discharge	24	61	30.4	20.0
Nasal obstruction	58	68	28.6 (injection)	64.0 (injection)
Fauces:				
Injected	73	6	73.9	68.0
Dry pharynx	41	Lymph follicles 33	39.1	32.0
Exudate	1		0	0
Chest signs:				
Rales	14	11	0	0
Rhonchi	20	29	0	0

* Commission on Acute Respiratory Diseases: Studies of the 1943 epidemic of influenza A. I. Introduction, Amer J Hyg, 1948, *48*, 253.

† Reference (7).

fever in servicemen was recorded as 3.6 days. In a closely similar military population of American troops, 1,264 cases of Asian influenza were observed by Bashore and others (5), and, in these, three days of pyrexia were found except when complications developed. The highest average temperature in 1937 was 101.2°F. and in the Asian cases in Cleveland, 58 per cent of adults reached a maximal temperature between 100 and 102°F. A close parallel existed at a residential boys' school in England during two epidemics, the one of Asian influenza in 1957 and the other of influenza B in 1959. The average duration of fever was slightly longer for the influenza B than for the A2 cases. Many of the boys were affected by both illnesses, but even the epidemic curves and attack rates were similar (6).

To return to table 3, the physical signs involving the face, conjunctivae, and the nose and throat show some variation from one epidemic to the next. Yet all agree in the occurrence in a varying percentage of patients of a flushed face, of glistening, sometimes weepy eyes, of a posterior pharyngitis devoid of exudate, and of a blocked or running nose. Added sounds in the chest are recorded with reluctance in uncomplicated cases of influenza by some observers. But the fact is that they are found at times even in previously healthy adults and *their absence from the majority of cases of Asian influenza* in many of the recorded outbreaks is perhaps a point of some importance.

To sum up, the symptoms and signs of the ordinary uncomplicated cases of influenza due to the Asian virus were remarkably similar to those found in epidemics due to influenza A and A1 viruses. Clinicians had no clue from the character of the ordinary disease of Asian influenza that they were dealing with an unusual strain of virus.

CLINICAL FEATURES OF THE CHEST COMPLICATIONS OF INFLUENZA

If a single feature which has distinguished the chest complications seen during influenza epidemics in the past twenty years were to be emphasized, I believe that the occasional occurrence of severe, often fatal, pneumonia associated with staphylococcal infection would be

chosen. It is difficult to estimate the frequency of such cases in the various epidemics, for many such patients never reach a hospital nor are they fully investigated. Also, the past twenty years have been ones of changing treatment and of a changing presentation of pneumonia apart altogether from influenza epidemics. In 1937 we recorded one case of staphylococcal pneumonia but knew of two others also occurring in London (7). In 1940–1941, Finland and co-workers (8) experienced an unusually large number of cases of staphylococcal pneumonia with varying clinical pictures during the influenza A epidemic in Boston and recalled the classical paper of Chickering and Park (9) on the role of the Staphylococcus in the 1918 outbreak at Camp Jackson. Wollenman and Finland (10) pictured the striking morbid pathology of the trachea and lung in such cases and pointed out that the findings of tracheobronchitis and hemorrhagic pneumonia closely resembled those of the 1918 pandemic (11). During the various epidemics of the influenza A1 era, many case reports were published in different countries which recognized the importance of staphylococcal pneumonia as a cause of fatal influenza, and our experience in Sheffield certainly agreed with this (12).

The Asian influenza epidemic resulted in the recognition all over the world of cases of influenzal-staphylococcal pneumonia. This is not to deny the numerically greater number of pneumococcal complications, but to stress the importance of the Staphylococcus in *fatal* cases of influenzal pneumonia. In the two largest series of fatal cases reported in the literature—namely, those from England, Wales, and Northern Ireland (13), and from the Netherlands (14*a*)—the Staphylococcus was found in 61 per cent of the 467 English cases and in 68 per cent of the 103 Dutch cases. In the English series it was the sole organism other than the virus in 47 per cent of cases. Similar experiences have been reported from Cleveland by Oseasohn and associates (15), from Boston (16), and elsewhere in studies of fatal cases.

Nevertheless, all groups of observers dealing with severe cases of Asian influenzal pneumonia recorded some cases often causing severe, and sometimes fatal, illness in which the lungs or sputum did not yield pathogenic bacteria. These apparently purely viral pneumonias, though uncommon, must be regarded as a new feature of influenza, for they had never been encountered in such numbers before in virologically studied epidemics. Moreover, the histologic studies of Louria and others from New York (17) and of Hers and colleagues in Leyden (14*b*) appear to have provided for the first time a description of the morbid pathology of pure influenza virus infection of the lower respiratory tract.

The second feature of lower respiratory tract complications of influenza in the past few years is the fact that they have often occurred in persons with pre-existing chronic disease of the chest or heart. Yet this is a point of view which did not attract attention at all, so far as I have been able to ascertain, during the 1918 pandemic. It was, however, stressed by Tyrrell (18) in his study of the pulmonary complications seen in Sheffield during the 1949 A1 epidemic of influenza. It has certainly been emphasized by the experiences of many observers during the Asian influenza epidemic. Thus, Walker and associates (19) studied 125 patients in Edinburgh with laryngotracheitis, bronchitis, or bronchiolitis and pneumonia. Seventy-three had previous chronic respiratory disease, mostly chronic bronchitis, pulmonary tuberculosis, bronchiectasis, or asthma, and fifty-two had no such history. In Dundee, a combined study group (20) found that patients with known cardiac disease, including mitral lesions, cardiac failure, or congenital heart disease, as well as chronic respiratory cripples, were severely affected by Asian influenza and often developed pneumonia. At Yale, much to my surprise, Petersdorf and coworkers (21) found that there were fourteen patients with chronic pulmonary disease and eleven with heart disease among ninety-one patients with influenzal pneumonia. Ten of the twenty-four females of childbearing age were pregnant and this was three to four times the expected proportion. In the Cornell series, reported by Louria and others (17), as many as twenty-one of the thirty patients with serious pulmonary complications had chronic heart or chest disease or were pregnant, and these writers put forward the view that mortality from influenza is more dependent on host factors than on the virulence of the virus. One is at least justified in stressing the special vulnerability of certain persons in the population previously not thought to be at special risk. The experience in Sheffield (table 4) supported this viewpoint. In saying this, however, one should add also that we have insuf-

TABLE 4

CITY GENERAL HOSPITAL, SHEFFIELD

PNEUMONIA, SEPTEMBER–OCTOBER, 1957*

Previous or Accompanying Conditions	Total		Staphylococcal		Non-staphylococcal	
	Number	Deaths	Number	Deaths	Number	Deaths
None	54	10 (19)	19	8 (42)	35	2 (6)
Chronic chest disease	61	15 (25)	13	7 (54)	48	8 (17)
Heart disease (rheumatic, hypertension, ischemia)	19	8 (42)	4	2 —	15	6 (40)
Pregnancy	6	1 —	2	1 —	4	0 —
	140	34 (24)	38	18 (48)	102	16 (16)

* Data from Robertson, L., Caley, J. P., and Moore, J.: Importance of *Staphylococcus aureus* in pneumonia in the 1957 epidemic of influenza A, Lancet, 1958, *2*, 233.

(Figures in parentheses indicate percentages.)

ficient data to support a conclusion that Asian influenza was necessarily more lethal to respiratory and cardiac cripples than other varieties of influenza virus infection. The fact, long known to epidemiologists, that the death rate from all causes of sickness rises during influenza epidemics stresses a contrary opinion.

UNUSUAL COMPLICATIONS OF ASIAN INFLUENZA

Finally, we must consider the possibility that Asian influenza produced certain unusual clinical manifestations or was followed by unusual sequelae. Three possible candidates exist, namely, obstructive laryngotracheitis, nervous complications, and cardiac complications.

Obstructive Laryngotracheitis

During the outbreak, several children with unusually severe obstructive laryngotracheitis were admitted to hospitals in Sheffield. Professor R. S. Illingworth drew my attention to this during the outbreak, and Diggle (22) recorded four cases. Obstructive laryngotracheitis is not a rarity in children. The unusual features of the Asian influenza cases were that they occurred in older children than is usual. Moreover, the obstruction was caused by viscid mucus which was difficult to remove and the symptoms were present at an early stage of the disease. Lindeman and Harböe in Norway (23), Walker and colleagues in Edinburgh (19), and Forbes in Melbourne (24) recorded similar experiences. The latter author found that 127 patients were admitted to the hospital with moderate or severe laryngeal obstruction. Seventy-five per cent of these were nine years of age or less; the course of illness was uninfluenced by antimicrobials.

Neurologic Complications

There has long been known to be an association between influenza and various neurologic syndromes. Leichtenstern (25) said:

> With a disease like influenza, which affects 50 per cent of the population, it is certain that numerous nervous and other diseases which occur during the course or after the epidemic will be attributed directly or indirectly, to influenza, although they would doubtless have appeared sooner or later in any case. Nevertheless, there can be no doubt that influenza has been the direct cause of numerous polymorphic diseases of the nervous system.

He also said that the pandemic of 1889–1890 had taught something new, namely, that influenza had striking neurotoxic effects compared with other infectious diseases. After the 1918 pandemic, neurologic phenomena following influenza were confused by the occurrence of the epidemic of encephalitis lethargica; and the relationship between these two diseases has, of course, never been settled.

I myself saw no nervous system complications at all during the 1937 epidemic, but encountered three or four instances in 1939. A relatively few cases of encephalitis, radiculitis, polyneuritis, and of the Guillain-Barré syndrome were recorded sporadically following influenza in the following years. Even in 1956, Miller and associates (26), in their extensive review of neurologic cases following acute infection, had nothing to say about influenza as a cause of encephalomyelitis. But before 1955, Sir John Charles (27) noted the occasional association in England between sporadic cases of encephalitis and influenza epidemics. In such cases, however, before 1957 no one had succeeded in showing that influenza virus was actually concerned in causing the nervous system lesions and many of the cases were of the post-influenzal demyelinating variety which is known to follow almost any acute infection.

The Asian epidemic appears to have led to a revival of interest in the neurologic sequelae of

influenza, and many references to these have now appeared in the literature. The clinical phenomena thus described are diverse. States of delirium or acute mania (28) and cases of encephalopathy with convulsions and coma have occurred in which abnormal electroencephalographic records have been obtained (29, 30). In addition to these, the more usual post-influenzal encephalomyelitis of the demyelinating variety, cases of polyneuritis, the Guillain-Barré syndrome, and of meningitis with lymphocytic exudate in the cerebrospinal fluid have all been attributed to Asian influenza. As many as thirty-six cases of influenza with encephalitis, meningitis, or myelitis were investigated by Herzberg and co-workers (31) in Frankfurt. In England and Wales (table 5) there seems no doubt that an excess of fatal cases of encephalitis secondary to influenza was reported during the Asian outbreak. Several fatal cases of acute onset of influenza with delirium, convulsions, and coma with or without upper motor neurone lesions or ataxia and a normal or abnormal cerebrospinal fluid were reported.

In the cases described by Flewett and Hoult (32) from Birmingham, Mastrogiovanni (33) from Naples, and Wells and associates (34) from Cardiff, virus strains of influenza A2 have been recovered from the cerebrospinal fluid or brain, usually at autopsy. The pathologists concerned have pointed out that such post-mortem recoveries of virus could have been due to contamination. On the other hand, Kaji and colleagues (35), working on fatal cases of Asian influenza in Cleveland, have demonstrated that virus can be recovered from spleen, heart, and other extra-pulmonary tissues. In view of the existence of acute hemorrhagic encephalitis and of lesions resembling those of other viral encephalitides (36) at autopsy in patients also exhibiting the tracheal and pulmonary lesions of influenza, I think it is reasonable to conclude that the neurologic lesions sometimes represent a true influenza virus encephalitis and at other times are due to a toxic encephalopathy. It is perhaps odd that no one has yet reported that the viruses recovered from human nervous tissue possess any neurotopic properties in animals. It is clear that much remains yet to be learned in this field.

Cardiac Complications

Reference has already been made to the complicating effect of influenza in persons with pre-existing cardiac disease. In persons free from such lesions, influenza has been reported as a cause of a variety of cardiac disturbances ranging from toxic effects to myocarditis and pericarditis. Finland and associates (37) reported two cases of infiltrative myocarditis during the influenza A era and a number of writers (15, 16, 38) have found similar lesions in severe cases of Asian influenza with pulmonary lesions. The rarity of such cardiac lesions must be stressed. It is probable that influenza of any type precipitates heart failure in middle-aged or elderly persons perhaps because of its toxic effect on the heart muscle. It is difficult to regard this as a specific effect of Asian influenza virus infection. Similarly the benign pericarditis cases described by Adams (39) were post-influenzal to influenza B as well as to Asian influenza. The general effects of influenza upon the circulation have been studied carefully by some of those here today (40, 41), and I shall leave it to them to take up the theme of the role of influenza virus in cardiac disease.

TABLE 5

ENGLAND AND WALES: DEATHS FROM INFLUENZA AND FROM ENCEPHALITIS SECONDARY TO INFLUENZA IN SUCCESSIVE PERIODS, JULY TO JUNE*

	Persons (All Ages)			
	1955–56	1956–57	1957–58	1958–59
Influenza	2,628	809	8,343	7,842
Influenzal encephalitis	2	1	30	19
Encephalitis per 1,000 cases of influenza	0.8	1.2	3.6	2.4

* Prior to 1955 only reported deaths from encephalitis were available for comparison, except in 1950–51. Nicol (Monthly Bull Min Health & Emerg Pub Health Lab Serv, 1952, *11*, 8) found that sixty-three deaths from acute encephalitis at all ages from July 1950 to June 1951 were associated with influenza in twelve instances. From influenza in all forms 15,809 deaths occurred, and the rate of encephalitis per 1,000 fatal cases of influenza was 0.75.

Other Possible Effects

Post-influenzal depression is a well-recognized sequel to influenza. It was not more prominent after Asian influenza than after other influenzal infections. Jaundice with raised serum bilirubin and fatty changes in the liver must be very un-

common during influenza. It was reported from India by Kapila and others (42), but was not encountered in most other series of cases elsewhere. A lesion of the kidney with tubular changes was reported by Beswick and Finlayson (43). Apart from albuminuria, influenza certainly does not cause any obvious effects on the kidney clinically, and the significance of the lesion is obscure. Probably a much more important effect is the possible induction of fetal abnormalities in pregnant women affected by influenza in the first trimester and noted in Eire (44). This is the first report of the sort suggesting that influenza can affect the fetus, and it is potentially a most important statement.

Summary and Conclusions

In conclusion, I would again like to refer to the general clinical resemblances between Asian influenza and the disease produced by the influenza A and A1 viruses. All in all the resemblances far outweigh the differences. Some of the phenomena, such as encephalitis and myocarditis which were in any case rare, may well have occurred in precisely similar form during previous epidemics but, because of the massive nature of the Asian epidemic, have then been recognized more clearly. But one cannot exclude the possibility that they were an expression of an attack by the virus on a largely unprotected community without previous experience of the Asian virus antigen.

Complications, when they occurred, were mostly of bronchitis or of patchy or segmental bronchopneumonia in previously crippled chest or cardiac invalids or in the elderly. They usually responded to chemotherapy directed against the commonest bacterial cause—the Pneumococcus. Staphylococcal cases which were often fatal are a reminder of the limited control yet afforded by antimicrobials against the combined viral-bacterial attack.

It is preferable to focus attention on the essentially benign character of Asian influenza rather than on its mortality rate. The question for control is rendered urgent by the exceptionally high attack rate experienced in all parts of the world. This morbidity is the present recurrent threat of influenza, but the mortality of future epidemics cannot accurately be foretold.

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General Discussion

Dr. David E. Rogers (Nashville, Tennessee): I am delighted to have the opportunity to supplement Professor Stuart-Harris' elegant summary of the clinical spectrum of influenza and its complications. I believe our experience with a selected group of patients may add depth to the picture he has sketched today.

During the influenza pandemic of 1957–1958, my associates and I had the opportunity to perform detailed studies on 30 patients hospitalized because of serious pulmonary complications of influenza.[1, 2]

Our experience with this group of patients led us to believe that three distinct syndromes could be characterized by the clinical history, the findings on physical examination, and microbiologic and laboratory data.

The most common clinical syndrome was that of bacterial pneumonia following influenza (table 6). This illness occurred in both normal adults and patients with underlying disease. Eight of 15 patients with this syndrome had significant antecedent car-

[1] Rogers, D. E., *et al.*: Trans Ass Amer Physicians, 1958, *71* (Supplement, p. 260).

[2] Louria, D. B., *et al.*: J Clin Invest, 1959, *38*, 213.

diopulmonary disease or pregnancy. Typically, these patients had influenza followed by a period of improvement lasting one to four days. There was then the sudden reappearance of illness, which in 10 of the 15 was ushered in by a shaking chill, increasing fever, pleural pain, and rusty, bloody, or purulent sputum.

These patients were acutely ill on admission to the hospital. Examination of the lungs revealed typical signs of local consolidation. Bacterial pathogens were readily culturable from the sputum, and the response to antimicrobials differed in no way from that seen in bacterial pneumonias observed during inter-epidemic periods. Mortality in this illness was low. One of 15 patients died of unassociated disease thirty days after recovery from influenza. Influenza virus was rarely isolated from throat washings, but a fourfold or greater rise in hemagglutinating and complement-fixing antibody was obtained in all instances.

In a second group of 9, the patients were believed to have a clinical syndrome produced by both influenza virus and bacterial infection acting in concert. Five had underlying heart disease. Here the onset of obvious lower pulmonary tract disease was early. Patients were more seriously ill, and there were many clinical features suggesting the influenza virus pneumonia syndrome I will subsequently describe. This illness pursued a stormy course, and 4 of the 9 patients succumbed to their acute disease process. In this group, both influenza virus and bacterial pathogens were obtained from throat washings, sputum cultures, and the pulmonary tissues of patients who died. The response to antimicrobials directed against bacterial pathogens isolated from sputum was often unpredictable and disappointing. It is our belief that influenza virus infection *per se* played a more important role in this process than has been generally recognized.

Lastly, a group of 6 patients developed an illness which we believe represented influenza virus pneumonia unassociated with complicating bacterial infection. This syndrome has not been well characterized in the past. Indeed, influenza virus infection as a fatal process was not widely accepted as a possibility prior to 1957–1958. This syndrome of primary influenza virus pneumonia may represent an increasingly important problem in this era of effective antibacterial agents, and the experiences of the recent pandemic have aided in defining its characteristics (table 7).

This illness arose in middle-aged patients with underlying heart disease. Rheumatic heart disease with mitral stenosis and/or insufficiency was present in 4 of 6 patients. Here, typical influenza was rapidly followed by increasing dyspnea, tachypnea, and cough productive of scant amounts of bloody sputum. Pleural pain was not a feature of this illness. On examination these patients were critically ill. All showed marked tachypnea and profound cyanosis. Pulmonary findings were those of poor air exchange. Diffuse rales and respiratory wheezing could be heard throughout the lungs, and signs of focal consolidation were absent. Bacteria could not be seen on sputum smears and no pathogens could be obtained on culture of sputum or blood, while influenza virus was readily isolated from throat washings.

The disease in these patients showed little or no response to antimicrobials, and their illness was characterized by increasing arterial oxygen unsaturation, rising arterial carbon dioxide, and death. Five of these 6 patients died with diffuse hemorrhagic tracheobronchitis and hemorrhagic pneumonitis from which influenza virus could be recovered in high titers. No bacteria could be recovered from the lung tissues or visualized on microscopic section. Chest roentgenograms of such patients showed a pulmonary picture compatible with acute pulmonary edema. Nevertheless, our studies to date suggest that this illness did not represent the simple pulmonary edema of cardiac failure.

TABLE 6

SYNDROME OF BACTERIAL PNEUMONIA FOLLOWING INFLUENZA

(15 Patients)

History:
1. Typical influenzal syndrome
2. Improvement for one to four days
3. Fever, chills, pleural pain, bloody sputum

Examination:
1. Local consolidation
2. Bacteria in sputum smears and culture (pneumococci, staphylococci, *H. influenzae*)
3. Response to antimicrobials
4. Low mortality (1/15)

TABLE 7

SYNDROME OF INFLUENZA VIRUS PNEUMONIA

(6 Patients)

History:
1. Age thirty to fifty, rheumatic heart disease
2. Typical influenza
3. No remission, increasing fever, dyspnea, scant bloody sputum

Examination:
1. Extreme tachypnea, cyanosis
2. Poor air exchange, diffuse findings, expiratory wheezing, no local consolidation
3. Scant bacteria on smear, no pathogens on culture
4. No response to antimicrobials
5. High mortality (5/6)

Venous pressure and blood volume determinations were generally within normal limits. Physical or electrocardiographic signs suggestive of myocarditis were absent. These patients did not respond to vigorous therapy for acute congestive heart failure, and the autopsy picture differed quite strikingly from that of simple pulmonary congestion. These observations have led to the conclusion that influenza virus infection can produce a characteristic and often fatal pneumonic syndrome in such patients. Indeed, in our experience, 50 per cent of the deaths observed were caused by this abacterial process in patients with previous heart disease.

It was initially our belief that underlying pulmonary hypertension and the precarious pulmonary fluid balance in such patients caused rapid exudation of fluid into the lung in response to influenza virus infection ordinarily tolerated without difficulty by normal persons.[3] Review of our own material and the accumulated experience of the 1957–1958 epidemic suggests, however, that pulmonary hypertension alone did not often result in this syndrome. Indeed, the relationship between rheumatic heart disease and fatal abacterial influenza seems impressive and specific.

Upon review of all fatal cases of influenza reported since 1933 in which virus has been recovered from the lung, it emerges that 25 per cent of the patients have died of a pneumonic illness unassociated with demonstrable bacterial pathogens[2–9] (table 8). Information on the presence or absence of underlying cardiac disease was available on seventeen of the thirty-five patients who died without obvious bacterial pulmonary infections. Thirteen of these seventeen patients had rheumatic heart disease. The mitral valve was involved alone or in association with other valve lesions in each instance (table 9). Thus, one cannot help but believe that rheumatic valvulitis represents a fairly specific precursor to fatal influenza virus pneumonia.

One gains the impression from the older literature that all types of pneumonia formerly produced high fatality rates in rheumatics. It seems possible that the altered cardiopulmonary dynamics in this group—significant pulmonary hypertension, high left auricular pressures, and the inability to increase cardiac output during the tachycardia associated with infection—may all contribute to the fatal pulmonary process associated with influenza infection in this group.

TABLE 8

BACTERIOLOGIC FINDINGS IN FATAL CASES OF INFLUENZA IN WHICH VIRUS WAS DEMONSTRATED IN THE LUNG

1937–1959

Total Cases, 143			
Bacterial (75%)		Abacterial (25%)	
Staphylococci	89		
Pneumococci	6		
Beta hemolytic streptococci	2		
H. influenzae	1		
E. coli	7		
Alpha streptococci	1		
Unknown	2		
Total	108	Total	35

TABLE 9

UNDERLYING DISEASE IN FATAL CASES OF ABACTERIAL INFLUENZA

Total, 35		
Underlying disease		Status unknown
Rheumatic heart disease	13	
ASCVD*	1	
"Myocarditis"	1	
"Normal"	2	
Total	17	18

* ASCVD—arteriosclerotic heart disease

DR. MAXWELL FINLAND (Boston, Massachusetts): Professor Stuart-Harris' theme of continuous change within the same general pattern is certainly consonant with our own experience with influenza in Boston[1] and undoubtedly with that of most other workers who have been intimately exposed to the influenza problem from the point of view of the clinician and laboratory worker for the past twenty or more years. There is not much of importance that I can add to his very well-rounded review and summary and the additional elaboration by Dr. Rogers of the clinical features of the uncomplicated disease and its important and serious complications and sequelae. I would therefore like to take this opportunity to review and reflect on some of our experiences with certain features of the dis-

[1] Most of the experiences mentioned have been aided in part by grants from the National Institutes of Health.

[2] FINLAND, M., *et al.*: J Clin Invest, 1945, *24*, 192.
[4] PARKER, F., JR., *et al.*: Amer J Path, 1946, *22*, 797.
[5] MAXWELL, E. S., *et al.*: J Clin Invest, 1949, *28*, 307.
[6] GILES, C., AND SHUTTLEWORTH, E. M.: Lancet, 1957, *2*, 1224.
[7] HERS, J. F. P., *et al.*: Lancet, 1957, *2*, 1164.
[8] PERRIER, C. V., *et al.*: Praxis, 1957, *46*, 1121.
[9] ROCK, J. A., *et al.*: JAMA, 1958, *166*, 1467.

ease in which we have been particularly interested at the Boston City Hospital.

The frequency and prominence of staphylococcal pneumonia in many localities during the pandemic of 1957 recalled the experiences of certain military installations where, during the 1918 pandemic, staphylococcal pneumonia was the major cause of fatalities associated with influenza. The first extensive experience with similar cases in which this association with influenza virus was established at the Boston City Hospital was in the winter of 1940–1941.[2, 3] In subsequent outbreaks, when equally intensive studies of the bacterial component and more detailed studies of the viral infections were carried out, the Staphylococcus did not seem to achieve similar prominence until 1957,[4, 5] although isolated cases or small numbers of cases, including fatal ones in which the influenza virus together with the Staphylococcus was implicated, were encountered in the intervening years.[6–8] Other severe staphylococcal infections were being encountered with increasing frequency throughout the entire period.[9] During these years the outbreaks of influenza usually occurred during the period of peak incidence of pneumococcal pneumonia, and the association of most of the pneumococcal pneumonias with influenza virus disease was the most frequent and could readily be established during the time of the occurrence of influenza.[6] The situation in the fall and winter of 1957–1958 and again in 1960 is somewhat different because of the relatively lower frequency with which pneumococcal pneumonias—in contrast to the much greater frequency with which severe staphylococcal infections—are being encountered both in the community and in the hospital during influenza outbreaks and in the intervals between them.[9]

The experience in 1940–1941 suggested the possibility that there was something quite specific about the relationship between the particular strains of staphylococci and of influenza virus that were then prevalent—something akin to the combination of the specific strain of influenza virus and of *Hemophilus influenzae* which Shope had earlier described in relation to fatal influenza in swine. In the latter's studies, it was possible experimentally to authenticate this specific potentiating combination for the swine. In 1941, Osler L. Peterson (unpublished data) in my laboratory tried to establish a similar relationship between the prevalent virus and the Staphylococcus. He used mice, but succeeded only in demonstrating that the Staphylococcus from the pneumonia patients was capable of producing boils on the dorsum of the hands of the experimenter, confirming the human virulence of the strain. Currently we may be dealing with any of several factors: strains of staphylococci with specific virulence, a heavy volume of staphylococci available for establishing infection in the presence of influenza virus injury, a virus that is capable of enhancing the susceptibility of the host to any virulent Staphylococcus or for some specific strain, and possibly other factors. In our own 1940–1941 material, Peterson was unable to identify any known property or combination of properties of the staphylococci from cases of influenzal pneumonia which distinguished them from strains isolated from other sources. In 1957–1958, at least as judged from phage types and antibiograms, the staphylococci from the pneumonias complicating Asian influenza virus infections showed the same differences as those staphylococci that were obtained from other sources before, during, and after the epidemic.

Influenza and myocarditis: In March, 1943, at a time when there was no awareness of any influenza virus infection in this community, we encountered a rapidly fatal case of staphylococcal pneumonia, and influenza A virus was readily isolated from the lung.[7] A month later we were confronted with a patient thought to have a nonbacterial pneumonia who died quite rapidly with congestive heart failure, shown to be due to acute extensive interstitial myocarditis. There were localized interstitial foci of nonbacterial inflammatory reactions demonstrated in the available sections of the lungs, and influenza A virus was isolated on two separate occasions by mouse passage of the lung suspensions.[10] Both of these patients were young women, twenty-six and thirty-four years of age, respectively, who had previously been in good health. During the epidemic of influenza A of the following winter, one of the patients who died in March, 1944, of acutely fatal nonbacterial hemorrhagic pneumonia was shown to have foci of the interstitial myocarditis; influenza A was readily and repeatedly isolated from the lung of this patient, but cardiac tissue had not been saved for this purpose.[6]

Following these experiences, we have been on the alert for cases in which the relation between the cardiac lesion and the influenza virus could be more

[2] Finland, M., *et al.*: Arch Intern Med, 1942, *70*, 183.

[3] Wollenman, O. J., Jr., and Finland, M.: Amer J Path, 1943, *19*, 23.

[4] Martin, C. M., *et al.*: AMA Arch Intern Med, 1959, *103*, 515.

[5] Martin, C. M., *et al.*: AMA Arch Intern Med, 1959, *103*, 532.

[6] Finland, M., *et al.*: J Clin Invest, 1945, *24*, 192.

[7] Parker, J. F., *et al.*: Amer J Path, 1946, *22*, 797.

[8] Finland, M., *et al.*: J Lab Clin Med, 1948, *33*, 32.

[9] Finland, M., *et al.*: JAMA, 1959, *170*, 2188.

[10] Finland, M., *et al.*: Amer J Med Sci, 1945, *209*, 455.

clearly established. Cardiac tissue has been studied histologically in all fatal cases in which influenza virus was implicated, and attempts to demonstrate virus in cardiac tissue were made in several cases in different outbreaks since 1944. Thus far we have succeeded in demonstrating influenza A1 virus from myocardial tissue in only one case. This was in a patient who was admitted to the hospital with symptoms and all confirming evidence of myocardial infarction that began during clinical influenza in 1953. This patient's lungs showed the characteristic findings of influenza virus pneumonia uncomplicated by bacterial infections, and influenza A1 virus was readily isolated from both pulmonary and myocardial tissues on two separate occasions a year apart. Histologically, however, the heart showed only the classical findings of a myocardial infarction and no evidence of any foci of myocarditis.[11]

In 1957, we had available the autopsy material from a patient fifteen years old with extensive myocarditis and minimal pulmonary lesions. The infection in this case occurred in August, about four months prior to the major epidemic in this area. Virus could not be isolated in our laboratory from the heart, lungs, and tracheal secretions, but specific fluorescence was demonstrated in the tracheal epithelium, and influenza virus was isolated from the lungs in another laboratory. Smaller foci of possible myocarditis were demonstrated during this year in 9 other cases among 30 autopsies of influenza-associated cases, but we failed to isolate virus from the heart or to demonstrate clearly specific fluorescence in the myocardial tissues of any of these patients.[4]

In view of all these findings, we have held some reservations about the relation of influenza virus to the myocardial lesions in any of our cases. We have, therefore, been particularly interested in the various reports of cases that have occurred during the 1957 pandemic and more recently, in which acutely fatal, nonbacterial pneumonia and myocarditis were reported along with isolation of the Asian influenza virus from the lungs and myocardial tissue. We have not encountered any late and clearly authenticated case of post-influenzal myocarditis such as was described clinically in the years immediately following the 1918 pandemic.

There could, of course, also be merely a coincidence of a case in which the influenza virus infection is authenticated and there is another cause of the myocarditis that could not be identified. The fairly clear demonstration that acute myocarditis could be caused by other viruses, notably Coxsackie B4, necessitates a thorough search for other viruses in acutely fatal cases of myocarditis even should they occur during influenza outbreaks or possibly even in a patient with a definite influenza virus infection.

I would like to bring up another point, although this one was remotely implied in my remarks about the complicating staphylococcal pneumonias. I refer to the question of viral interrelationships insofar as influenza is concerned. The epidemic of late 1943, in Boston at least, followed very closely a period of high prevalence of primary atypical pneumonia, particularly of the variety which is associated with the development of cold isohemagglutinins.[12] At that time we were struck with the fact that pneumonias of this variety were not encountered during the height of the influenza epidemic and were infrequently encountered for several months thereafter although they had been observed quite frequently in the preceding year or two. We have had moderate numbers of cases of such atypical pneumonias in subsequent years but not in a similarly high incidence in our area since then, but we have rarely encountered cases in which influenza virus or specific antibodies to it have been demonstrated during periods of high prevalence of this disease in a patient in whom a nonbacterial pneumonia was also associated with the development of cold isohemagglutinins. Since we have not made complete studies of incidence, it is not possible to state whether this is merely what might be expected from the relative frequency of the occurrence of these diseases.

Turning now to another variety of probable viral disease, there have been at least three occasions during outbreaks of characteristic influenza virus infections when there was also an unusual prevalence in the same community of a febrile illness associated with abdominal pain and diarrhea and sometimes with vomiting, but without respiratory symptoms. The patients frequently have chills or chilly sensations and generalized weakness. Enteric bacterial pathogens were not found in these cases. On each occasion we had the opportunity to study throat washings and serum from the acute and convalescent stages from several such patients, but have failed to find evidence of influenza virus infection in any instance, even in patients in whose families influenza virus infections were authenticated at the same time. In view of the reports of simultaneous occurrence of cases with severe gastrointestinal symptoms during a current epidemic on the West Coast and also in and around Boston, I shall be interested to hear whether any specific virus has been looked for and identified or whether influenza virus has been clearly implicated as the only viral infection of some of the recent cases. I regret to say that, because of other preoccupations, we have not

[11] Finland, M., and Barnes, M. W.: Amer J Hyg, 1955, *62*, 1.

[12] Finland, M., *et al.*: J Clin Invest, 1945, *24*, 458.

availed ourselves of the opportunity to make these observations in recent cases in our own community.

Finally, I have been unable to formulate any clear picture of the relation of herpes simplex infections to febrile cases of influenza virus infections uncomplicated by such other specific infections as those caused by pneumococci or streptococci which are known to be accompanied or followed by exacerbations of herpes simplex. Several years ago in preparing monographs on both streptococcal and staphylococcal pneumonias, I was struck with the fact that herpes simplex was not mentioned in any clinical accounts of the staphylococcal pneumonias, whereas it was frequently mentioned as occurring in pneumococcal and hemolytic streptococcal pneumonias. There are, of course, persons who are known to have exacerbations of herpes labialis following each attack of the common cold, or at least following many of them, and the problem of multiple infections with influenza and other respiratory viral diseases including the common cold is another one worthy of exploration.

Professor Stuart-Harris mentioned the first fatal case of verified influenza A associated with staphylococcal pneumonia that he and his colleagues described in 1937 and also some of the others that have been described since then. He indicated that the uncomplicated fatal viral disease had never been encountered in the same numbers prior to the 1957 pandemic. Of course, the patients described by Wollenman and myself[9] all had superimposed staphylococcal pnuemonia and one case also was complicated by the hemolytic Streptococcus. In 1946, with Parker and others, we described the pathology of 5 verified cases of influenza A pneumonias, 2 of which were uncomplicated by any bacteriologic or histologic evidence of a bacterial component; and others were also encountered later.[11] At the time when we were studying the earlier patients, we succeeded in locating the description and histologic section of a case studied by the late Frank B. Mallory in 1918, in which bacteriologic and histologic studies failed to reveal any pathogen in the gross description, and histopathology was identical with what we observed in one of the patients whom we studied in 1944. Dr. Wollbach was also able to find a similar case among the autopsies that he had performed at the Chelsea Naval Hospital during the 1918 pandemic, and of course there were the two cases from the same pandemic described by E. W. Goodpasture.[10] Among the thirty-two cases of influenza-associated fatal cases that were described by Martin and associates from the Boston area in 1957 pandemic,[4] fifteen were not accompanied by any demonstrable bacterial component, eleven were accompanied by staphylococcal infection, and only six with infection by other organisms. This latter group may be deceiving with respect to the proportion of such cases, since nearly all of those with the pneumococcal pneumonias complicating influenza recovered with proper antimicrobial therapy whereas, of those accompanied by staphylococcal infection, more than half ended fatally.

It should also be mentioned that we have not yet encountered a fatal case of parainfluenza B pneumonia, i.e., without a bacterial component.

Dr. Theodore E. Woodward (Baltimore, Maryland): I am not an expert on influenza, but I was interested in Dr. Finland's comment that they had not observed a continuing type of myocarditis in Boston, and I cannot say emphatically that we have either. However, we observed a young lady with acute influenza during the 1957 outbreak who had the pulmonary manifestations that Dr. Rogers related and unmistakable signs of myocarditis with gallop rhythm and all of the other well-known clinical features. She recovered and left the hospital with some signs of a weakened myocardium, if you will permit such a statement. She returned about eight months later desperately ill again with unmistakable signs of myocarditis and subsequently died on the day of this re-admission. I might add that at the time of the preliminary admission, virus was isolated from the throat washings and she ultimately developed the serologic reactions confirming that the illness was influenza. I regret to say that we were unsuccessful in obtaining an autopsy.

Dr. Harold B. Houser (Cleveland, Ohio): I want to make some comments about our experience when our influenza study group was in Chile (JAMA, 1958, *167*, 290). There were greatly increased numbers of pneumonias because of the widespread epidemic there and the abnormally high prevalence of respiratory infections. Twenty-six of the 49 autopsy cases studied had no other contributory cause of death such as a chronic illness or heart disease. It appeared then that death was occurring in supposedly normal people. I think the potentiality for death in normal people with influenza is certainly present. The other point is that 25 per cent of the fatal cases had the clinical signs of a purely influenzal type of pneumonia. I was interested in seeing Dr. Rogers' report from the literature of about 25 per cent, which of course corresponded very closely with what we found in Chile. However, in this 25 per cent we did not observe any association with rheumatic heart disease. The 25 per cent of fatal cases in which death was due primarily to influenza occurred apparently in normal people. Therefore, rheumatic heart disease was not a necessary underlying cause of death in the Chilean

[10] Goodpasture, E. W.: Amer J Med Sci, 1919, *158*, 863.

population. Another point of interest was that, from observations made in several hospitals, we found only a slight increase in the case fatality rate due to pneumonia. There was a great increase in the number of admissions, but the case fatality rate itself did not increase greatly. The increased mortality from pneumonia, therefore, was due primarily to the greater number of cases rather than lack of successful treatment.

Dr. Thomas Francis, Jr.: I want to comment about one of the clinical manifestations which Dr. Stuart-Harris mentioned. In my experience it is quite new to the picture of influenza. I refer to the acute laryngitis of the severe kind that apparently has been prevalent during this year and two years ago, but especially this past year. This has not just been in children, because there have been fatalities in adults where tracheotomies have been required. In my memory, going back to 1918 and since, this laryngitis is an entirely new phase in the picture of influenza. One other thing, I have never been impressed with the sore throat in influenza, but in these instances of laryngitis, it hurt to swallow.

Dr. George G. Jackson (Chicago, Illinois): I wanted to comment briefly on the problem of staphylococci in these pneumonias. It seems quite certain from analysis of data that Staphylococcus is important in the fatal cases of post-influenzal pneumonia. During the 1957–1958 epidemic, we made some attempt to ascertain the ecology of the Staphylococcus among all patients with serologic evidence of influenza, and among another group with pneumonias that were presumably primarily bacterial. From our data there was no propensity for a person with influenza to acquire staphylococci in a hospital, and those who entered the hospital carrying a strain of Staphylococcus lost it at the same rate as those people who did not have serologic evidence of influenza. This was true of the group of patients who had clinical influenza before the pneumonia and entered the hospital with staphylococci in their sputum, as well as those who had a rise in titer during their hospitalization and presumably were in the phase of acute influenza. It seems that, if one follows the ecology, and this was compared with office groups of patients also, there was no over-all propensity of these patients with serologic evidence of influenza to acquire staphylococcal infection although the organism was prominent in the fatal cases of post-influenzal pneumonia.

PHYSIOLOGIC ABNORMALITIES

ALTO E. FELLER, *Chairman*

A STUDY OF THE RESPONSE OF THE CARDIOVASCULAR SYSTEM TO ASIAN INFLUENZA[1,2]

GEORGE E. BURCH, JOHN J. WALSH, AND WILLIAM MOGABGAB

Introduction

The foreknowledge of the approaching pandemic of Asian influenza permitted the design of multidisciplinary studies of the effects of this virus in man. Our particular interest was primarily directed to the cardiovascular system. This paper is a report of our studies; more detailed communications are available elsewhere (1–12). The disease arrived in New Orleans, the first major city in the continental United States to be so massively involved, in the summer of 1957. This early appearance of the epidemic precluded the use of all available facilities as originally planned. The short life of the local epidemic and the high incidence of illness among technical and medical personnel further limited our studies.

Materials and Methods

Thirty-four patients, ranging in age from twelve to seventy (mean, twenty-nine) years, were included in this series, all but 4 of whom were less than forty years of age. The distribution by age, sex (12 men and 22 women), and race (9 white and 25 Negro) was to be expected from the nature of the study and the patient population of Charity Hospital. Although the diagnosis of Asian influenza during the height of the epidemic was readily established on the basis of clinical data alone, laboratory confirmation was also obtained in 30 of the 34 patients. Throughout hospitalization, all patients were observed at four-hour intervals, when temperature, pulse, respiratory rates, and systolic and diastolic blood pressures were recorded.

The patients were selected on the following bases and divided into two groups for convenience of presentation:

Group I consisted of 30 patients: (*1*) Twenty with the typical clinical picture of early influenza on admission to the outpatient department of Charity Hospital had all or most of the investigative procedures mentioned performed on them. (*2*) Ten patients had been hospitalized because of the severity of their pulmonary infection or because they did not have adequate home care. Routine electrocardiographic studies revealed abnormalities in all, and virologic confirmation of the diagnosis was established in all.

Group II consisted of 4 patients who experienced severe cardiovascular complications of influenza. These will be discussed in a separate section of this report.

In addition to routine clinical and laboratory examinations, the following studies were obtained on all patients: fluid and electrolyte measurements, adrenocortical function, serial standard electrocardiograms, spatial vectorcardiograms, digital rheoplethysmograms, renal and hepatic function, bronchoscopic biopsies, and cytologic examinations. The magnitude of the clinical problems is apparent when it is realized that the first outpatients with influenza seen at the Charity Hospital during the early days of August, 1957, increased within four weeks from a few dozen to well over 1,000 a day. The epidemic reached its peak about the first week in September, 1957, and within a week or ten days patients suitable for study were no longer available. Overtaxed hospital facilities and illness among the professional and technical personnel interfered with the studies.

Group I

Clinical Features

The history and physical findings in these patients were typical of influenzal infection. The only exceptions were encountered in those patients with complications of one sort or another. It is of interest that in 6 (30 per cent) of these patients, alterations in breath sounds were recorded which could best be described as exaggerated vesicular breathing. Other observers also noted the frequency of this finding in uncomplicated influenza and were able to associate it with influenzal infection uncomplicated by pulmonary consolidation. The course of illness was such that the febrile state persisted for 4.4 days in uncomplicated influenza and for 6.7 days in those patients with complicating pneumonia. Slightly more than one-third of the patients exhibited a diphasic or "M" type of febrile curve.

[1] From the Department of Medicine, Tulane University School of Medicine; the Charity Hospital of Louisiana; and the Cardiopulmonary Research Laboratory of the United States Public Health Service Hospital, New Orleans, Louisiana.

[2] Aided by grants from the United States Public Health Service.

All patients were extremely uncomfortable, but only 2 appeared seriously ill. Both had pneumonia. One of these, an elderly female with amyotrophic lateral sclerosis and arteriosclerotic heart disease, had considerable difficulty in evacuating respiratory secretions. Expectorants, bronchodilators, and wetting agents relieved this difficulty. The second severely ill patient was a young male with staphylococcal pneumonia who showed rapid and marked improvement after two days of intensive antimicrobial and supportive therapy. Uncomplicated Asian influenza was unaffected by antimicrobial therapy. Patients with complicating pneumonia responded well to the antimicrobials, usually within twenty-four to forty-eight hours after the initiation of therapy. Thirty per cent of the 30 patients experienced bacterial complications of Asian influenza. These consisted of pneumonia (in 6), purulent bronchitis (in one), purulent conjunctivitis (in one) and beta hemolytic streptococcal pharyngitis. All responded to conventional therapy with no sequellae.

Associated conditions: There was one patient in the nephrotic stage of chronic glomerular nephritis whose renal disease was not altered by the influenzal infection. The single patient with amyotrophic lateral sclerosis, as previously mentioned, had difficulty in evacuating respiratory secretions.

There were 3 patients with underlying cardiac disease. Two were elderly patients with arteriosclerotic heart disease who suffered from pneumonia as well as influenza. Although one of these exhibited multiple premature ventricular contractions at the height of illness, neither patient experienced worsening of his cardiovascular status. A third patient who had long-standing compensated rheumatic mitral insufficiency and cardiomegaly had no cardiac difficulties incident to the influenzal infection. Similarly, a patient with bronchial asthma did well, although early in the course of the influenzal infection he was moderately dyspneic and had generalized rhonchi and wheezes which responded to expectorants and bronchodilators.

Three patients were pregnant at the time of their influenzal infection. One of these experienced a complicating pneumonia. All 3 did well and carried their pregnancies to the expected dates, delivering normal, full-term infants.

Laboratory studies: Extensive laboratory studies were performed in these patients. In no patient was renal or hepatic function altered by uncomplicated influenzal infection. The erythrocyte sedimentation rate was elevated in almost all patients. Leukocytosis usually indicated the presence of a bacterial complication of the viral infection. Routine agglutination determinations (heterophile, typhoid O, typhoid H, paratyphoid A, Proteus OXK, *Brucella abortus*, and cold agglutinins) were within normal limits as were all studies involving the cerebrospinal fluid. The serum glutamic oxalacetic transaminase was unaffected by influenzal infection. Bacteriologic studies were generally unremarkable, positive sputum cultures being obtained from only 2 of 6 patients with pneumonia (*Klebsiella pneumoniae* and Staphylococcus). However, it should be noted that these studies were of the sort obtained in routine hospital patient care and were not extensively or specially directed.

Serial determinations of 17-hydroxycorticoids were within normal limits in all patients. Similar studies of 17-ketosteroids revealed 3 patients in whom there was a mild transitory increase in excretion of these compounds at the height of illness. Transitory minimal decrease in serum and urinary osmolarity occurred at the height of illness in about 20 per cent of the patients so examined. This group also showed minimal decrease in sodium excretion at the same time.

Throat washings obtained from all 30 patients were cultured in embryonated eggs. Fourteen such specimens were found to contain the Asian strain of influenza virus. Paired sera, as determined by hemagglutination-inhibition antibody studies, were diagnostic of the Asian strain in 27 of these 30 patients. In addition, the virus was recovered from culture of tracheal material obtained at bronchoscopic biopsy in 2 of 9 patients.

Cardiovascular Responses

Pulse rate: In health, at normal body temperature, the pulse ranges from seventy to eighty beats per minute. Commonly it is accepted that each degree of elevation in body temperature results in a pulse increment of approximately ten beats per minute. In the current series, whenever the pulse rate was more than ten beats per minute slower or faster than the limits predicted on this basis, bradycardia or tachycardia was considered disproportionate.

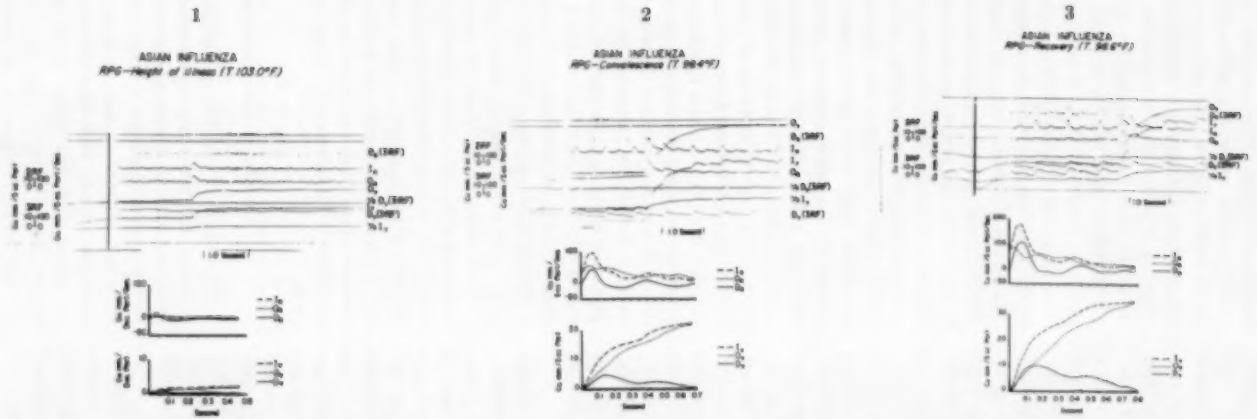

FIG. 1 (Left). Rheoplethysmogram of right index finger (2RF) and right middle finger (3RF), recorded simultaneously, in a patient with Asian influenza. I_V, O_V, and D_V represent volumes of inflow, outflow, and difference between inflow and outflow, respectively, for 2RF. Similarly, I_R, O_R, and D_R represent rates of inflow, outflow, and difference between rates of inflow and outflow, respectively, for 2RF. D_V and D_R for 3RF are also shown. Simultaneous time course curves, showing temporal relations of the volumes and rates of digital blood flow, indicate vasoconstriction of digital circulation. The upper sets of tracings are actual recordings. Enlargements of the rate and volume curves for a single pulse cycle in the upper sets of tracings have been transposed to the two lower groups of curves.

FIG. 2 (Center). Rheoplethysmogram of the patient shown in figure 1, taken during convalescence. Time course curves indicate that the digital vessels are less constricted. See legend of figure 1 for explanation of symbols.

FIG. 3 (Right). Rheoplethysmogram of the patient shown in figure 1, taken during recovery. Time course curves reveal normal digital vascular tone, with considerably more rapid rates of digital blood flow. See legend of figure 1 for explanation of symbols. (*Reprinted through the courtesy of the Editor, Annals of Internal Medicine (1958, 49, 502).*)

Bradycardia: Two-thirds (20) of the 30 patients experienced at least one episode of disproportionate bradycardia. There was a total of fifty-four episodes, all occurring within forty-eight hours after admission to the hospital and almost invariably limited to febrile periods. During such episodes the median pulse deficit was eighteen (range twelve to sixty).

Tachycardia: Forty per cent (12) of the patients experienced disproportionate tachycardia for a total of thirty-eight episodes. Pulse excess ranged from fourteen to thirty-eight, with a median of twenty beats per minute. Disproportionate tachycardia was noted only during convalescence. Of interest was the finding that 10 of the 12 patients experiencing disproportionate tachycardia also were noted to exhibit disproportionate bradycardia.

Blood pressure: Transitory elevation (10–20 mm. of mercury) of the systolic blood pressure was noted in 5 patients during the first one to two days of hospitalization, at a time when anxiety and pyrexia were most pronounced. Two of these 5 patients also showed a simultaneous elevation in diastolic pressure of similar magnitude.

In only one instance was hypotension noted. This phenomenon occurred during the first twenty-four hours of hospitalization of a young man severely ill with staphylococcal pneumonia. During the period in question, diastolic pressure varied from 30 to 60 mm. of mercury, the systolic pressure remaining in the region of 120 mm. of mercury. Thereafter, blood pressure ranged from 100–110 systolic and 60–70 diastolic in mm. of mercury.

Respiratory rate: Only 6 (20 per cent) of the patients exhibited any significant alteration in respiratory rate during the period of observation. In this group the respiratory rate was approximately 50 per cent greater during the first seventy-two hours than during the remainder of hospitalization (27–30 versus 18–20 respirations per minute). Only 4 of these 6 patients complained of dyspnea, and 3 of the 6 had pneumonia. One patient exhibited slight cyanosis on admission and none required oxygen.

Circulatory dynamics: Venous pressure and circulation time were normal in all 12 patients in whom such determinations were made.

Rheoplethysmograms: Twenty patients, 16 of whom had laboratory confirmation of the diagnosis of Asian influenza, were subjected to rheoplethysmography at the height of illness, during convalescence, and after recovery. All recordings were made while the patient was resting in a hospital bed in a room with controlled atmospheric temperature and humidity (78°F., 46 per cent relative humidity). Examples are given in figures 1–3.

Fifteen patients (75 per cent) experienced decreased digital blood flow during the height of illness, with reduction in magnitude of the pulse wave and of the alpha deflections. With clinical improvement, digital blood flow increased, returning to normal levels on full recovery. During the period of vascular constriction, vessel response to stimuli was normal but of decreased magnitude. There was a gross correlation between severity of illness and degree of digital vasoconstriction. In only one instance was there a greater rate of digital blood flow at the height of illness.

Electrocardiograms: Of 20 patients with Asian influenza subjected to serial electrocardiographic study, 15 manifested abnormalities (75 per cent). On the other hand, only 10 of 36 additional influenza patients had abnormal routine electrocardiograms (28 per cent). In other words, serial electrocardiography yielded almost a threefold greater incidence of abnormalities than two routine electrocardiograms.

The most common abnormality was alteration in the T wave, which was noted in 23 patients. An example is given in figure 4. In 9 of this group the T wave was inverted or diphasic in one or more leads. In the remainder the T wave was significantly reduced in amplitude or was isoelectric. These alterations were limited to or most prominent in precordial leads V_4 through V_6, although in half of the patients, similar alterations were present in the standard leads as well. Generally, abnormalities were most prominent at the time of admission to the hospital and disappeared with convalescence, occasionally within twenty-four hours. There was one instance each of sinus bradycardia, minimal ST segment depression, prominent P waves, and right-axis deviation. The above-described changes were transient.

Spatial vectorcardiograms: Using the equilateral tetrahedron as a reference frame, serial spatial vectorcardiograms were recorded in 20 patients. An example is given in figure 5. In 4 patients the records were within normal limits. In the remaining, distortions of the QRS sÊ loop

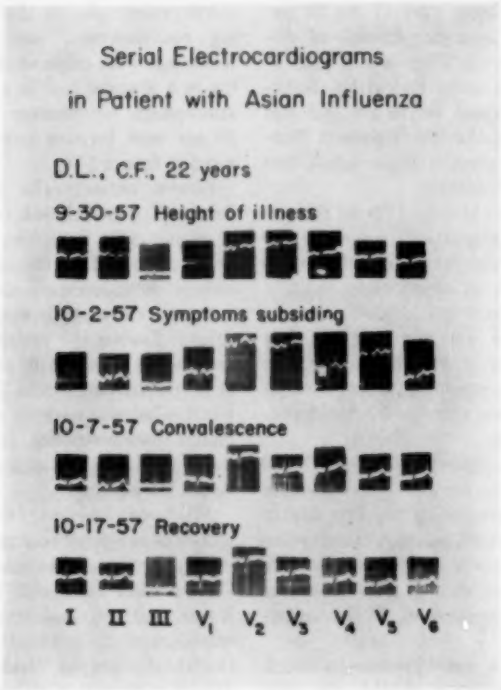

FIG. 4. Alterations in T waves in patient, 22 years of age, with Asian influenza at height of illness promptly disappeared with convalescence. (*Reprinted through the courtesy of the Editor, Annals of Internal Medicine (1948, 49, 502).*)

resulted in traces that were not as smooth in contour as are usually encountered in healthy young adults. These abnormalities may represent alterations in depolarization produced by subtle injury not detectable by conventional electrocardiography. In 6 patients these abnormalities disappeared with convalescence. In the remaining 10 the absence of change on serial examination is compatible with pre-influenzal configuration or residua of influenza.

In 4 patients at the peak of illness the afferent limb of the QRS sÊ loop exhibited slight arching similar to that observed in diaphragmatic myocardial infarction. These abnormalities disappeared with recovery. The sVCG suggested slight right ventricular hypertrophy or dilatation, or both, in these 4 patients.

Endobronchial Pathology

Bronchoscopy with bronchial biopsy was performed in 9 patients at the height of illness. The pathologic changes are shown in figures 6–12. Grossly, there were various degrees of edema, hyperemia, congestion, and bronchorrhea. In 2 patients the virus of the Asian strain of influenza A was recovered on culture of the biopsy material. Microscopic changes were essentially those of irregular epithelial desquamation with scattered areas of a transitional type of metaplasia. Cellular nuclei were pyknotic and of variable size and shape. The basement membrane and the lamina propria were thickened and edematous. Vascular engorgement and inflammatory infiltrates were consistently noted. Frequently the elastic tissue was edematous, fragmented, and exhibited an increased affinity for eosinophilic stains. As a rule, pathologic changes became more intense as one progressed distally in the tracheobronchial system.

Electron microscopy of bronchial epithelium obtained three days following the onset of symptoms revealed discrete particles, approximately

22 mμ in average diameter, dispersed throughout the cytoplasm and surrounding cytoplasmic vacuoles. Large aggregates of similar particles were confined to the nuclei of the affected cells. These particles have not been previously reported, and their precise nature is obscure at this time.

Group II

There were 4 patients in group II who experienced clinically significant cardiovascular complications of Asian influenza. Two were young females with rheumatic mitral stenosis who experienced severe left ventricular failure incident to the influenzal infection. A third young, gravid female, who was at term, suffered from pulmonary edema and pneumonia during her episode of Asian influenza. There was no preceding or succeeding illness, and the viral infection was the only obvious cause for the severe illness from which the patient suffered. The fourth patient was also a young female who experienced simultaneously with Asian influenza a severe episode of rheumatic pancarditis, complicated by congestive heart failure. All of these patients exhibited a fourfold or greater rise in hemagglutination-inhibition antibody titer against the Asian strain of influenza. There is little doubt that influenza precipitated the severe cardiovascular symptoms in the first 3 patients. In the last patient, the relationship was more tenuous and may not have existed at all. The rheumatic activity may have been due to an undemonstrated streptococcal infection complicating influenza or to a smoldering rheumatic process which became manifest as a result of the influenzal infection.

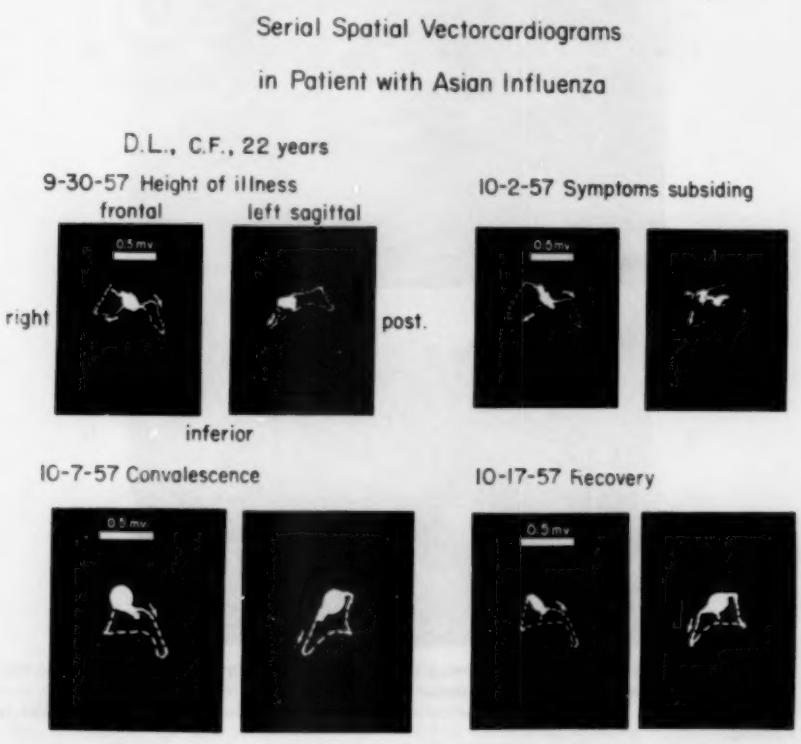

Fig. 5. Serial spatial vectorcardiograms of patient whose corresponding electrocardiograms are shown in figure 4. Note irregularities in contour and diaphragmatic arching of afferent limb of QRS sÊ-loops, which became less prominent with convalescence. (*Reprinted through the courtesy of the Editor, Annals of Internal Medicine (1948, 49, 502)*)

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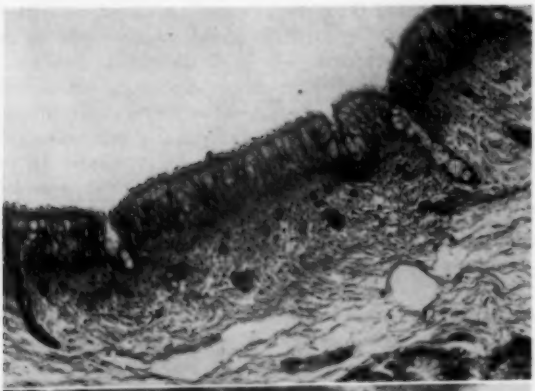

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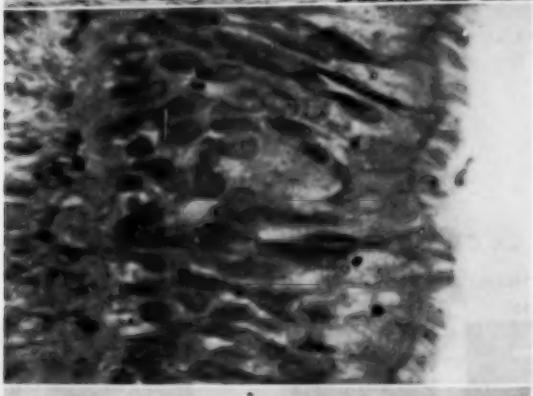

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FIG. 6 (Top). Low-power view, showing normal human respiratory epithelium. Note the typical pseudostratification of the columnar cells.

FIG. 7 (Center). With greater magnification of the same section, the abundance of cilia is striking.

FIG. 8 (Bottom). Tracheal epithelium one day after the onset of symptoms. Desquamation of epithelium in areas to the basement membrane has occurred. There is "hyalinization" and thickening of the basement membrane, a sparse mononuclear infiltrate, and eosinophilia of the elastic tissue.

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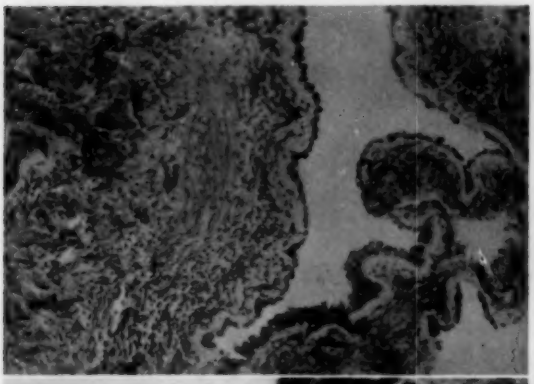

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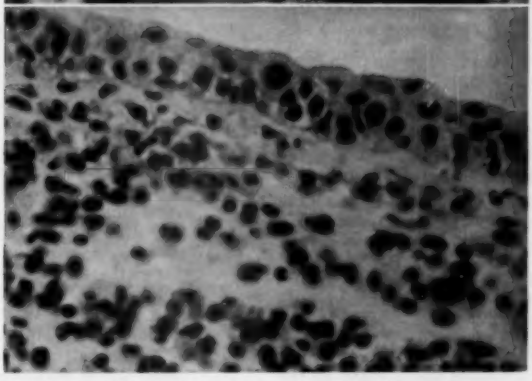

FIG. 9 (Top). Tracheal epithelium three days after onset of symptoms showing various degrees of epithelial desquamation. The previously noted changes in the basement membrane are again apparent.

FIG. 10 (Center). A high magnification of bronchial epithelium showing hyperchromatic nuclei of variable size and shape and "hyalinization" of the basement membrane.

FIG. 11 (Bottom). Bronchial epithelium six days after onset of symptoms, showing squamous metaplasia, consisting of two to three stratified layers of cells with pleomorphic and hyperchromatic nuclei. Intercellular bridges are not visible, and there is little or no tendency toward surface flattening.

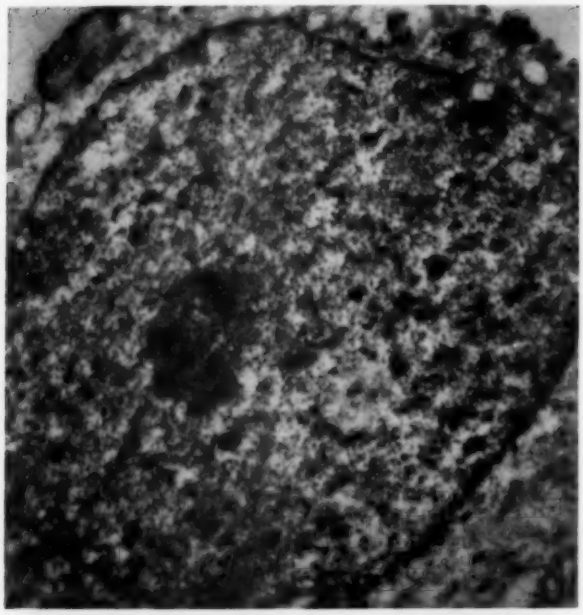

FIG. 12. Electron photomicrograph of bronchial epithelial cell three days after onset of symptoms. Discrete nuclear condensations are visible, dispersed throughout the nucleus. There is no evidence to suggest that these cells are dying or manifesting a nonspecific response to injury. No such phenomenon was observed in the control specimens.

Finally, it could have represented a spontaneous bout of rheumatic fever in a patient with a significant rise in hemagglutination-inhibition antibody titer incident to an asymptomatic influenzal infection.

SUMMARY

Thirty patients with Asian strain A influenza were intensively studied by clinical and laboratory methods. In 26 of these patients, specific diagnostic tests substantiated the diagnosis; the usual reservations in the diagnosis of viral diseases applied. Attention was primarily directed to the effects of influenza on the cardiovascular system. At the height of illness digital vasoconstriction and abnormalities of the T wave in the standard electrocardiogram and the QRS sÊ loop in the spatial vectorcardiogram were noted in approximately three-fourths of the patients. These alterations usually disappeared with convalescence. Clinically significant cardiovascular complications were not encountered in this group. However, 4 patients experienced cardiovascular complications of considerable magnitude. In each instance the diagnosis of influenza was confirmed by standard laboratory techniques.

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General Discussion

Dr. Park W. Willis, III (Ann Arbor, Michigan): Our studies were similar in scope to those of Dr. Burch and colleagues, but involved patients in a lower age range.

During the 1957–1958 Asian influenza epidemic we examined 105 young adults hospitalized at the University of Michigan Health Service with clinical and serologic diagnoses of influenza. Our primary concern was to determine whether influenza had a discernibly significant effect on the heart or peripheral vascular system.

The medical history of 58 of these subjects disclosed no symptoms suggesting cardiac abnormality. During the acute illness, postural dizziness was noted by more than one-third of the group, and syncope was reported on four occasions. Frequent physical examinations during the acute illness revealed no evidence of cardiac involvement. Approximately 15 per cent of the patients had relative bradycardia early in the acute illness. Blood pressure measurements obtained during hospitalization in 92 cases, when compared with those obtained during convalescence, showed no evidence of hypotension as a common event.

To study further the cardiac effects of influenza, electrocardiograms were taken in 57 cases at frequent intervals during acute illness and during convalescence. Disturbances of rhythm were noted in 11 patients during hospitalization, and in 7 after recovery (atrial and ventricular premature beats, wandering of the pacemaker within the SA node, sinus arrhythmia with a nodal escape beat, A-V nodal rhythm, complete interference dissociation, and first-degree A-V heart block). Disturbances of repolarization, manifested by T-wave changes during the acute infection, were found in 34 patients. Twenty-five of these had slightly low T waves; 5 had very low, probably abnormal, T waves; and 4 others had inverted, definitely abnormal, T waves. With one exception, the T waves returned to normal during convalescence. The exact significance of these findings is uncertain, in view of the known lability of the T waves and the frequency of T-wave abnormalities in infectious diseases. The findings do suggest the possibility of myocardial involvement in influenza.

To obtain further information about possible direct effects of influenza on the myocardium, frequent determinations of serum glutamic oxalacetic transaminase and lactic dehydrogenase levels were obtained in 61 persons. These enzymes are present in high concentration in heart muscle cells, and serum levels are often elevated in patients with active myocarditis. Sustained significant elevations of lactic dehydrogenase were seen in 2 patients during acute illness. One of these patients had a definitely abnormal electrocardiogram. The other, unfortunately, had no electrocardiogram recorded. Serum enzyme levels in convalescence were uniformly normal. These findings suggest a possible relationship between elevated serum enzyme levels and electrocardiographic abnormalities in influenza. Further studies of this type are needed and are in progress.

If the electrocardiographic and enzymic changes observed are related to an effect of the virus on the myocardium, it would seem possible that viremia might precede this event. To examine this question, virus isolation was attempted in sixty-two blood specimens obtained from 8 patients approximately eight to ninety-six hours after the onset of symptoms. No virus was recovered from these samples. It appears that viremia does not occur commonly after the first day of clinically apparent infection.

In a group studied with tests of vascular reactivity, no consistent abnormality in peripheral vascular responsiveness could be demonstrated during illness or in convalescence.

Determinations of cardiac output in one patient and of total blood volume in 3 patients during illness were normal. The roentgenographic examination of the heart was normal in 38 patients during the acute phase of the infection. Careful questioning did not reveal a significant element of post-influenzal asthenia or weakness in this group of young and otherwise healthy persons. No tendency for hypotension to occur during convalescence was evident.

Although further studies are clearly indicated, the results of this investigation suggest that influenza may occasionally have a significant effect on the heart of an otherwise healthy young adult. This would not appear to be a common event.

Dr. Robert R. Wagner (Baltimore, Maryland): Dr. Feller, I must confess I am here under false pretenses: first, because I am no physiologist and, second, I am really here to introduce Dr. Edward Hook, who has been doing far more interesting studies than anything that I can report. The question we have set ourselves to answer is whether the influenza virus can actually interact in some way with blood vessels. This seems important whether or not one believes that the virus is confined invariably to the respiratory tract or whether on occasion it may get out of the respiratory tract and into the blood stream.

One of the first and simple things we did was to find out whether the influenza virus could, in fact, adsorb onto the surface of vascular endothelium. I would like to report the results of a study done by a young medical student who put a canula into the portal vein of a mouse and another one into the inferior vena cava and perfused the liver with a known amount of virus, using an infusion pump at a constant rate and measuring the virus content of the effluent. It was found that there was a very constant and rapid adsorption of the virus onto the surface of the hepatic endothelium. Some elution of virus occurred when the liver was warmed up; or, when the experiment was performed at 37°C., elution occurred at a much faster rate. We think that this is probably a specific reaction, for when the receptor surface of the endothelium is destroyed by pretreating with one-hundredth molar periodate, the virus does not adsorb onto the surface of the endothelium.

Second, we have been interested in the fact that the influenza virus can infect tissues other than the lung of experimental animals. Dr. Stuart-Harris has shown that his neurotropic WS strain is capable of infecting the brain. We have used this strain to do some studies with infant mice. It was found that the age of the animal makes a great deal of difference in the capacity of the tissues to support multiplication of the influenza virus. The virus was found to be capable of multiplying in the liver of the infant mouse over a period of time. In addition, the influenza virus was observed to multiply in skeletal muscle as well as in heart, brain, kidney, and other tissues. We wondered what was common to these tissues that would allow the virus to grow. It seemed that blood vessels were the one thing that was common to these tissues. However, the infant mouse did not turn out to be a very satisfactory experimental animal in which to study the intimate relationships between the virus and the vascular endothelium. Studies were then made using chick embryos. Particular attention was given to hemorrhagic encephalopathy that occurs in chick embryos, and this has been studied by Dr. Hook.

Dr. Alto E. Feller: Thank you, Dr. Wagner. This discussion will be continued now by Doctor Hook.

Dr. Edward W. Hook (New York, New York): As Dr. Wagner said, we have been interested in the pathogenesis of the extensive vascular lesions that develop in chicken embryos infected with the NWS or neurotropic variant of the WS strain of influenza A virus.

After fourteen-day-old chicken embryos are inoculated intravenously with small infecting doses of NWS virus, the titer of virus increases rapidly and at similar rates in a number of different tissues, including brain, liver, and skeletal muscle. The tissues of infected embryos reveal no pathologic changes during the first fourteen hours after inoculation. Fourteen to twenty-four hours after infection a generalized vascular reaction develops that is characterized by swelling of endothelial cells and marked engorgement and dilatation of small blood vessels. Capillary rupture begins to occur twenty-four hours after infection and leads to multiple small hemorrhages. In fourteen-day-old embryos vascular rupture occurs predominantly in the brain, producing extensive hemorrhagic encephalopathy. The reason hemorrhages occur predominantly in the brain is obscure.

We believed from the onset of our interest in the pathogenesis of this lesion that the early diffuse damage to small blood vessels in the absence of changes in parenchymal cells suggested that the virus might actually multiply in endothelial cells. This hypothesis, which was first advanced by Burnet and Fraser a number of years ago, has been confirmed by utilizing the fluorescent-antibody technique to localize viral antigens in infected cells. Specific, equally intense staining of vascular endothelium in brain, liver, heart, lung, muscle, skin, and feathers was first detected when the EID_{50} of the tissues obtained a level of 10^4 to 10^5. As the titer of virus increased, small blood vessels were outlined by almost uninterrupted lines of fluorescence. Extravascular antigen was not observed until after capillary rupture, when scattered nodular areas of fluorescence were detected at sites of hemorrhage.

We believe that these observations are of interest. They indicate that vascular endothelium is the primary site of NWS virus multiplication in chicken embryos inoculated intravenously. These studies also lead us to wonder if the same sort of phenomenon might occur with other viruses and in other hosts.

(*Note:* A series of photomicrographs was presented to illustrate the histopathology of NWS virus infection in chicken embryos.)

PHYSIOLOGIC ABNORMALITIES OF INFLUENZA[1]

Lessons Learned and Future Needs

DAVID P. EARLE

In 1957 I foolishly accepted an invitation to serve on a committee to consider the management of Asian influenza and its complications. As a consequence I have been trapped into speaking to you about "Lessons Learned and Future Needs" in regard to the abnormal physiology of this disease. This is embarrassing since I am undoubtedly the only speaker on this program who cannot present data of his own. Not even the title is my own.

Nevertheless, excellent pathologic (1–6) and clinical data (7–10) on severe influenzal infections are available both in the literature and in today's discussions. Recently, several groups (7–9, 11) have begun physiologic studies in influenza.

The most I can do for you is to pick out from these various sources several observations that may help to delineate the important problems for the clinical physiologist. Much of what I have to say is based on the work of the speakers on this afternoon's program. In the absence of specific therapy the most promising approach to management of patients severely ill with influenza is a thorough knowledge of the disturbed physiology that leads to death. Several examples among infectious diseases of how such an approach has been worthwhile might be quoted. One of the earliest and most dramatic examples of this was recognition in 1732 of the importance of salt and water loss in the diarrheal stools of cholera. Adequate replacement reduced mortality rates from 60 to less than 5 per cent (12). In fact, therapy based on knowledge of the abnormal physiology of cholera is so effective that little need for chemotherapy exists.

What have we learned about fatality due to influenza? We do not have to be very subtle to define the broad limits of this problem. Autopsy studies reveal that most fatalities in influenza are associated with extensive pneumonia. Often the pneumonia is due to secondary invasion by bacteria. In these instances, therapy with appropriate antimicrobials appears reasonably effective.

The situation in primary influenzal pneumonia, however, is quite dramatically different. That nonbacterial primary influenzal pneumonia exists can be doubted no longer. Several elegant pathologic studies during the 1918–1919 pandemic, especially those of Goodpasture (2) and Winternitz (1), certainly suggested this possibility. Subsequently, other reports (3, 8, 13) appeared describing fatal influenzal pneumonia with high virus levels in the lungs but without bacterial invasion. Within twenty-four hours of onset the patient is desperately ill with high fever, severe dyspnea, extremely rapid and shallow respirations, and hypoxia. Death typically occurs within a few days. Many but by no means all victims of fulminating influenzal pneumonia have pre-existing heart or pulmonary disease, or are pregnant. These associations demand explanations which should yield clues to abnormal physiologic mechanisms leading to death.

Simple clinical observation and the very characteristic pathologic features of severe influenza direct attention to extreme difficulties in respiratory gas exchange. One look at the lungs of a patient who has died of primary influenzal pneumonia gives one a rather hopeless feeling. How can anything be done for someone whose lungs are so congested that they weigh two to five times normal and whose alveoli are so drowned in fluid with the same protein content as serum (14) that arterial oxygen saturation may be only 35 to 40 per cent (8, 11, 15)? Even when breathing 100 per cent oxygen, the arterial saturation of these patients may remain below normal (8, 11, 16). But nothing in the pathology of influenzal pneumonia suggests that complete recovery is impossible.

Despite the obvious nature of the pulmonary difficulties, practically no observations on the physiologic abnormalities are available beyond those that can be inferred from simple observation. In 1919 Stadie demonstrated arterial oxygen unsaturation in thirty-three patients with influenzal pneumonia (15). Louria and colleagues (8) and Herzog and colleagues (11) confirmed this in the 1957–1958 outbreak, and added data on arterial blood pCO_2, bicarbonate, and pH. Except for a handful of observations on the effects of intermittent positive pressure breathing

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apparatus (8, 11) and of a carbonic anhydrase inhibitor (8), nothing else is known about this dramatically abnormal physiology. I am no expert in the field of pulmonary physiology, but I strongly suspect that much important data could be collected. And, further, therapeutic techniques undoubtedly could be developed that would tide the patients over their acute period of severe respiratory gas exchange problems. Louria (8) demonstrated that intermittent positive pressure breathing with the Bennett machine and with 100 per cent oxygen could return the arterial oxygen saturation of even the most severely ill patient to normal. Unfortunately, only one of six patients with primary influenzal pneumonia could tolerate the procedure for a significant period of time. This patient was the only survivor. Time does not permit going into the details of possible solutions of this problem, which involves increased arterial pressure of carbon dioxide which could perhaps be controlled by proper use of carbonic anhydrase inhibitors or by intravenous injections of the buffer tris-(hydroxymethyl-aminomethane), or by the design of the respirators and techniques that could be tolerated by the tachypneic patient. Proper use of tracheotomy (17) represents another useful advance. I strongly suggest that we interest our pulmonary function colleagues in these problems.

But all deaths are not necessarily due solely to respiratory anoxia. Another major field that warrants extensive physiologic study in influenza is the vascular system. Although I would very much like to know what happens to cardiac output, the term "cardiovascular" was purposely avoided. That "myocarditis" may be a sequela of influenza has been suggested more than once (7, 18); but that myocarditis accounts for any significant number of fatalities in influenza is unlikely. However, some evidence suggests that abnormalities of the pulmonary and peripheral vascular systems may be very important in severely ill patients. Aside from "medical" shock, the most direct evidence of this has been the demonstration by Burch and his colleagues (7) that digital arteriolar constriction was present in fifteen of twenty patients with influenza of variable severity. Aside from the observations already presented this afternoon and sporadic measurements of venous pressure, circulation time, and blood volume, practically no precise information about the behavior of the vascular system in influenza exists. Many of us who have suffered even a moderately severe attack of "flu" have reason to suspect that abnormal peripheral vascular physiology may contribute to our misery and to post-influenzal debility. Available techniques for studying general and local vascular flow and resistance, cardiac output, and direct observation of small blood vessels are feasible and relatively simple. Such observations, especially if made serially in severely ill patients, should yield information of basic importance.

Although of less practical importance, other areas such as the nervous and endocrine systems, the kidneys, and fluid and electrolyte metabolism are begging for quantitative observation in *severe* influenza, and in other acute infectious diseases.

So far, only relatively gross physiologic disturbances, amenable to study by techniques developed in the first half of this century, have been discussed. Looking into the future, I sincerely hope that application to influenza of more advanced techniques now being developed for study of abnormal pathophysiology at the cellular and biochemical levels will not be so sadly neglected. Perhaps such techniques will elucidate factors concerned with the localization of the influenza virus in cells and how it produces cellular damage. The earlier discussions of Drs. Wagner and Hook represent elegant examples of this approach. What metabolic derangements does the infection induce in the host? Are there toxins? Many other questions could be put to the experimental test.

Summary

In conclusion the following statements seem pertinent. Abnormal pulmonary and circulatory functions are undoubtedly responsible for the majority of deaths in primary influenzal pneumonia. Almost no precise data on the disturbed pulmonary and circulatory physiology of influenza exist. Techniques for the collection of such data are not only available but also could easily be applied to the study of even severely ill influenzal patients. The above remarks apply to many other acute infectious diseases.

In the few exceptions in which the disturbed physiology of an infectious disease has been carefully studied, the almost invariable result

has been improvement in the management of the patient and usually a significant reduction in mortality, whether or not specific antimicrobials or chemotherapy are available.

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General Discussion

Dr. Rodney Beard (Palo Alto, California): One thing that would be helpful would be to have Dr. Walsh and Dr. Willis put their electrocardiographic findings in some better perspective for many of us. They have indicated a high incidence of change in different perimeters as representing cardiac disfunction. It would be very helpful to me and others to be able to relate these findings in influenza cases in terms of the incidence of these electrocardiographic abnormalities in other acute febrile illnesses. In other words, my question really is: Are these changes of major moment in influenza or are they merely phenomena that occur during the course of any acute febrile disease process?

Dr. John J. Walsh (New Orleans, Louisiana): Generally speaking these changes are those which we see in infectious diseases to a variable extent. At one end of the disease spectrum is tuberculous myocarditis, which is quite a rarity, and at the other are some of the rickettsial diseases and other viral diseases in which the majority of patients will show electrocardiographic changes. In most of these instances, we could not attach clinical significance to the changes. In fact, we really don't know exactly what significance to attach to them. We have seen them and are still trying to interpret them. Of particular interest are the alterations, however minor, in the QRS complex. The QRS is not as prone to change as is the T wave. In most of the instances we see in everyday practice, the T wave alterations are due to noncardiac or nonspecific factors. The observations that were of particular interest to us were the alterations in the QRS complexes, the definite but limited digital vascular constriction revealed by rheoplethysmography, the instances in which there was either an abrupt shift from right to left avis deviation or from a prominent to a normal T wave with improvement, both of which would suggest some increased load on the right side of the heart. I don't know that I have an answer to your questions.

There is one other thing that I had meant to mention and had forgotten. The clinical criteria of myocarditis are nebulous, to say the least. Generally we look for gallop rhythm, hypotension, disproportionate tachycardia, systolic murmur of a

transitory nature, or an alteration in the first sound —the description of which, I think, depends upon the imagination of the observer—usually it is called "squashy" or "mushy." We particularly look for these symptoms, as I said, but I don't think we can attach any great significance at this point to the disproportionate tachycardia as indicative of myocarditis. Of the 30 patients, there was one patient who, as I mentioned, exhibited transitory hypotension and a transitory systolic murmur. There was another patient who also had a transitory systolic murmur, and there was a third patient who had multiple extraventricular contractions. These were the only three instances in which there was even one clinical indication of myocarditis, other than changes noted by instrumentations which I previously mentioned.

Dr. Park W. Willis, III: I would like to say that I agree completely with the things that Dr. Walsh has said. To the T wave changes we observe we ordinarily would give absolutely no attention; we consider them changes that would be expected in an acute infectious disease in the presence of fever.

Dr. Theodore E. Woodward: I believe the problem of myocarditis is a nebulous one also. Clinically we encounter it, and realize how little we know of it. I believe here is an opportunity for some groups who have observed patients with influenza, as one specific disease, to begin a long-term follow-up study of such patients, particularly those who have had some changes at the time of acute infection, and see whether a relationship can be established in some ways five, ten, fifteen, twenty years hence. Perhaps this is a beginning of something we can pinpoint later on.

Second, I would like to ask Dr. Walsh a question. Were any direct observations made of the peripheral blood vessels in the patients that were studied?

Dr. Walsh: No. No observations of that sort were made.

Dr. Jonas E. Salk: The suggestion that we learn more about physiologic abnormalities poses a problem, particularly since the clinical cases during epidemics occur during a period of time usually when all clinicians are busy. This raises the question whether or not the experimental uncomplicated human viral infection might not provide appropriate clinical material for making such observations, rather than the severe cases associated with bacterial complications. Influenzal asthenia following infections of the more common garden variety has made people wonder whether or not the syndrome doesn't, in part, resemble a mild endocrine disease and whether or not there might be some adrenal abnormality induced temporarily. On the other hand, the question has arisen in connection with studies on the reaction to influenza vaccine as to the possibility of a delayed hypersensitivity mechanism which contributes to the asthenia. It is possible that another model for experimental studies could be the use of very large doses of influenza vaccine followed by detailed physiologic studies.

Dr. E. N. Silber (Chicago, Illinois): I have hesitated to comment up until this point, but at Michael Reese Hospital in Chicago, pathologist Dr. Otto Saphir and some of us clinicians have been studying myocarditis for quite some time. There are many aspects of this problem which, I think, make it difficult to talk about myocarditis and influenza and other viruses. One of them is that it depends on how carefully you study the heart. Dr. Saphir has emphasized this. Because of his interest, he studies the heart rather exhaustively. He emphasizes that one may miss lesions when only three or four blocks of tissue are taken and studied by ordinary techniques.

It has been our clinical experience that the electrocardiogram is not a very helpful tool, particularly during the acute course of a febrile disease, for the reasons that I will mention. There are many things going on that may affect the T wave and AV condition and so on, so that the electrocardiogram is not always an accurate tool. In addition, like other organs, the heart has such a tremendous reserve that symptoms do not readily appear in acute myocarditis. For this reason we have focused on an end point of heart disease, which I think no one will question, and that is heart failure.

If the heart fails in the absence of pre-existing heart disease, we conclude that it followed the acute infection. Everyone, we believe, would agree that this is the best end point for making a diagnosis of myocarditis. Now, interestingly enough, it has been our experience that heart failure does not commonly occur during the acute phase of influenza or other viral diseases. Frequently, however, there is a latent period, after a minor or obvious influenzal infection, during which the patient will be clinically well for one or more weeks, just as in rheumatic fever, and then presents himself in congestive heart failure. If I am not mistaken, Dr. Finland originally reported in 1945 one of the two patients who ran such a course. Because of the latent period, all too often the heart failure is recognized as a brand-new disease. The relationship then to the previous infection is not recognized, so that we don't talk about post-influenzal or post-infectious myocarditis, but such cases are described as idiopathic heart failure, and so on.

It is my own feeling that post-influenzal myocarditis is much less rare than it is thought to be generally. By taking careful histories it is often revealed that patients, in the absence of pre-existing heart disease, often develop transient or even intractable signs of heart failure following infection. I have been hesitant to mention these observations,

for their documentation at present is not too good in terms of proof. However, heart failure cases are related to viral infections, although one cannot isolate the viruses because they have long since disappeared from the blood. The time interval is such that the serologic antibodies have again returned to normal or near normal, making them difficult to interpret. However, I think that one can, by clinical inference alone, consider that these are viral without further documentation. It is our impression —and something which we are going to work on to get documentation—that post-influenzal myocarditis is much less common than it is generally thought to be.

PATHOLOGY

ALTO E. FELLER, *Chairman*

BROAD ASPECTS OF THE PATHOLOGY AND PATHOGENESIS OF HUMAN INFLUENZA[1, 2]

J. F. Ph. HERS AND J. MULDER

We now know that influenza is caused by a virus that can infect the ciliated epithelial lining of the respiratory tract of human beings and certain animals. The broader aspects of the cytopathology of the respiratory epithelium in experimental animal influenza has been well described in the literature (1–11). More recently, it has been possible to confirm the existence of similar phenomena in human beings (12–16) provided that autopsies were performed early and certain precautions were taken in order to avoid artefacts. These recent findings in human beings were in part confirmation of the work of Winternitz and co-workers during the pandemic of 1918, reported in their classic monograph of 1920 (17). The change of the ciliated epithelium into a stratified layer of cells which appears in later stages of the disease had been mentioned by Widal as early as 1899 (18) and had been observed by many investigators in 1918 (17, 19–21).

In the acute stage of the disease the ciliated epithelium degenerates and desquamates, but one or two rows of basal replacement cells remain as a final covering of the basement membrane. Very probably these cells do not support the growth of virus. In our material, a stratified epithelial layer was found to exist in 4 cases, about nine, fourteen, sixteen, and eighteen days after the beginning of the disease. The disappearance of ciliated cells with the preservation of the basal cell layer has been observed in the trachea and bronchi of all of our virologically confirmed cases, and we know now with certainty that this destruction may be distributed quite focally and that it is independent of coexistent bacterial tracheobronchitis.

The actual process of cell destruction has been studied in histologic sections (14, 15) and this study has been aided recently by the work of Negroni and Tyrrell (22) on the microscopic details of influenzal cellular damage in tissue cultures of calf kidney cells. In these cultures, vacuolization of the cells seems to be the first microscopic alteration that can be recognized, and this is followed by a shrinkage of the cells with pyknosis and fragmentation of the nuclei.

In the human being, the microscopic degeneration of the ciliated cells should also be studied in cytologic smears, using common staining methods (13) and the fluorescein-labeled antibody technique (first applied by Liu (23)). In addition, phase contrast microscopy is helpful in comparing the pictures obtained by fluorescent microscopy with the normal appearance of the same cells or tissue.

The cytopathic changes seem to develop in the fashion described by Negroni and Tyrrell (22), but a conspicuous finding is that, when death occurs early in the disease, the ciliated cells in certain areas often appear damaged at an earlier stage than that of severe shrinkage. Very frequently one finds cells which have rounded or irregular cell bodies with swollen oval nuclei, often situated transversely in the cytoplasm (figures 1–4). These cells display no gross vacuolization or severe nuclear damage. Often the cilia are clearly visible. In this stage the cells desquamate. Occasionally however, the microscopic cell damage looks similar to the pictures described by Negroni and Tyrrell, but nuclear fragmentation is not common (figure 5). In sections it is often very difficult to decide between vacuolization of cells and intercellular spaces which may have been caused by shrinkage of the cells or rupture of vacuoles (figure 6). Small shrunken cells or nuclei are often seen phagocytosed by ciliated cells (*see* figure 2), which has also been beautifully described by Hotz and Bang (24) in ferrets, using electron microscopy. In pandemic Asian influenza, precisely the same pictures were seen as in seasonal influenza.

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In the future more investigations must be carried out by means of histochemical methods and electron microscopy. So far, however, there is ample direct microscopic evidence that influenza virus infection causes severe damage to the epithelium of the respiratory tract. This results in an epithelial wound, which facilitates secondary infection by bacterial respiratory pathogens, the most dangerous of which are the pyogenic cocci.

Much more difficult is the problem of the pathology and pathogenesis of human influenza virus pneumonia, and it is here that the experience with Asian influenza has extended our knowledge considerably. One of the present authors (14) has shown in eighteen virologically confirmed fatal cases of seasonal influenza A and A1 with coexistent bacterial infection (observed in the period 1941–1956) that the lesions in the epithelial lining were present in the respiratory bronchioles in only one case and that there apparently were no microscopic signs of influenza virus pneumonia.

Upon examining one hundred and forty-eight confirmed fatal cases of Asian influenza, it was found that in many instances there was damage to the epithelial lining of the respiratory bronchioles (25). In thirty of these cases without coexistent bacterial inflammation, a peculiar form of focal pneumonia was present which could be readily recognized to be identical with the pneumonic lesions found in certain cases by American pathologists in 1918 and considered by them to be true influenzal pneumonia caused by an unknown agent (17, 20, 21, 26–30). In the early stage of the disease the gross microscopic lesions are: (*1*) severe hyperemia and broadening of the alveolar walls with interstitial leukocytic infiltration; (*2*) alveolar hemorrhage; (*3*) capillary thrombosis with increased focal leukocytic exudate observed in 1918 by Le Count (27), Goodpasture (26), MacCallum (20), and Winternitz and associates (17), which can be clearly demonstrated by staining with hematoxylin-phloxin-tartrazin by Lendrum's method; (*4*) alveolar ducts (dilated) and alveoli covered with plasmatic exudate—the so-called hyaline membranes —(showing a positive periodic-acid-Schiff reaction), first found in 1918 by Oberndorfer (28) and by Wolbach (30) and especially studied by Goodpasture (26). In late stages peculiar epithelial proliferations are found in the respiratory bronchioles, growing into adjacent alveoli but also covering many alveoli in a single monolayer. In this stage fibrosis of the lung tissue begins to appear.

It must be emphasized that the investigator of 1957 was in a far better position to study pure influenza virus pneumonia than were his colleagues of 1918, for there is little doubt that in 1957 bacterial superinfection was depressed in many cases by the massive use of antimicrobials. This is illustrated by the fact that Goodpasture (26), who was looking for fatal cases not showing bacterial infection, managed to find only two examples for study.

In attempting to understand the pathogenesis of pure influenza virus pulmonary lesions, our attention was focused on the microscopic aspect of the alveolar cell lining. As a tentative hypothesis we argued that, if the peculiar pneumonic lesions were due to air-borne influenza virus infection, the first site of damage would be the alveolar cell lining, since this is probably the only pulmonary tissue that is able to support the growth of virus. The vascular lesions might then be considered either as secondary to the denuding of the alveolar walls from their protective covering or as the consequence of viral infection of the endothelium. Basically, the same explanation had been offered in 1920 by Winternitz (17), who emphasized the resemblance between the pathology of influenzal pneumonia and war-gas damage of the airways and lungs. If this hypothesis is correct, it should be possible to demonstrate cytopathic changes in the alveolar cells early in the disease. There is in fact very suggestive evidence that these changes are present. In most of the cases, by use of both cytologic and histologic techniques, very curious cell types were found, either still attached to the alveolar wall or lying within the exudate in the alveoli. The cells are characterized by small round nuclei showing a striking pyknosis or coarse particles of chromatin at the nuclear membrane. Often the nuclei are situated eccentrically in the cell. Fragmentation of the nuclei may occur. The cytoplasm tends to become denser and more eosinophilic. Vacuoles are common. The cells may become very small in size. They are often seen phagocytosed by macrophages. The alveolar walls become denuded of alveolar epithelium. Alveolar histiocytes (macrophages) may show the same changes, but nuclear fragmentation seems to occur more frequently and perhaps earlier in those cells than in alveolar cells (figures 7–19). It is quite remarkable that MacCallum (20) described

what was almost certainly the same type of cell in the alveolar exudate of thirteen of forty-four cases of influenzal pneumonia of 1918, but he remained uncertain of their origin.

That these cells are apparently derived from alveolar cells supporting the growth of virus is suggested by the following observations: (*1*) The type of cell may be attached to the alveolar wall and is not seen within the structures of this wall or in blood vessels. (*2*) There exist morphologic transitions between normal alveolar cells and this type of cell. (*3*) On the sites where this type of cell is abundant, normal looking alveolar cells are lacking or are present in small quantity, and vice versa. (*4*) They do not show a positive peroxydase reaction. (*5*) We were able to demonstrate in 2 recent cases of influenza virus A2 pneumonia (one associated with mitral stenosis and pregnancy, and the other with aortic valvular disease) that in cytologic smears as well as in freeze-dried sections, alveolar (septal) cells, alveolar histiocytes (macrophages), and the alveolar "lining" may show a strong specific fluorescence. In both cases specific fluorescence could be demonstrated in small, most probably alveolar, cells with small round nuclei, especially also when they were seen phagocytosed by alveolar histiocytes (figures 20–32). The vascular endothelium did not show fluorescence.

In influenza virus pneumonia in animals, the occurrence of a similar process may be assumed. In cytologic smears of infected mouse lungs the normal septal epithelium is partially replaced by very peculiar, often shrunken cells, showing nuclear pyknosis and often a striking nuclear fragmentation. It was demonstrated in smears that they may contain alkaline phosphatase, which is absent in the epithelium of the bronchi and bronchioli and alveolar histiocytes of mice (31). In freeze-dried sections of infected mouse lungs many alveolar (septal) cells and the alveolar "lining" show a specific fluorescence (figure 33). In stained sections severe cytopathic changes may also occur in most alveolar cells. They have probably been frequently mistaken for "mononuclear" wandering cells or, in the presence of nuclear fragmentation, for leukocytes. Exactly the same cytopathic effects in the alveolar lining are encountered in the so-called toxic influenzal pneumonia of mice, induced by the aspiration of high-titered egg fluids of strains of virus which are not lung-adapted (31).

Thus, although many more investigations are necessary, microscopic evidence makes it reasonable to assume that influenza virus pneumonia does exist and that it is caused by viral attack on the alveolar cell lining. The vascular damage, including the forming of hyaline membranes (which is probably the cause of death) may be a phenomenon secondary to the destruction of this lining.

It is of interest to note that by means of simple microscopic techniques it can be demonstrated that bronchotropic virulence and pneumotropic virulence of human strains of influenza viruses (A1) in mice represent two different, most probably genetic, properties. This is shown by the fact that in early passages of egg-line virus, mice show extensive viral cell damage in the bronchial tree without coexistent foci of pneumonia. What is generally called adaptation of a human strain of influenza virus to the mouse lung is most probably the induction into this strain of new properties enabling it to grow in a more or less new type of cell of the same host, namely the alveolar cell (31).

Since pure, fatal influenza virus pneumonia in men was apparently rare during the period 1933–1956 (only one case, reported by Parker and associates (32), seems to fulfill the criteria), a most important question is whether the Asian virus displayed an enhanced genetic pneumotropic virulence for the human being. This question cannot be answered with any certainty as yet for the following reasons: (*1*) The possibility of viral "toxic" lesions of the alveolar cell lining must be considered, associated with the fact that the majority of the human population was deprived of protecting antibody. Another factor in this matter may be the insensitivity of the virus to neutralizing nonspecific inhibitor, which might be present in human lung tissue. (*2*) It is generally accepted that unknown host factors often favored the appearance of influenza virus pneumonia in 1957. Influenza virus pneumonia has been found especially in patients with chronic congestion of the lungs due to heart disease (and associated with a peculiar hyperplasia of alveolar cells) in patients with kyphoscoliosis, in those who have undergone a pneumonectomy, and in pregnancy (25, 33–38) (table 1). (*3*) Fatal Asian influenza virus pneumonia was rare in 1957 and can roughly be estimated to have occurred in the Netherlands in only one of 4,000 cases of

TABLE 1

TYPES OF HISTOPATHOLOGIC LESIONS IN 148 VIROLOGICALLY CONFIRMED CASES OF ASIAN INFLUENZA

Virus lesion in respiratory tract Virus lesion in lung Bacterial inflammation	1 4%	2 20%	3 5%	4 40%	5 31%
Preexisting disease:					
Kyphoscoliosis		1		1	
Tuberculosis	1	1*	2		1
Emphysema and chronic bronchitis	1	2	1	8	2
Anthracosilicosis		1		3	1
Mitral stenosis		10†		1	
Aortic "	2	1			
Myocardial fibrosis		5		5	2
Other heart-disease		1			1
Multiple sclerosis				1	
Epilepsy				1	1
Idiocy				4	1
Other neurological disease			1	1	
Other disease	1	2		3	6
Pregnancy		1			1
Previously healthy	1	5	3	31	30

* Associated with pregnancy.

† 4 cases associated with pregnancy.

(This table is used through the courtesy of the Editor of The Lancet)

clinical influenza, including cases of coexistent secondary bacterial infection. (4) Asian influenza virus strains have not shown a high primary pneumotropic virulence in mice and ferrets in contradistinction to freshly isolated strains of Swine A virus, obtained from field cases in North American swine (Shope (39) and personal communication). So far this problem seems to us to be still unsolved, and we must confess that, even with the Asian strain of virus in our laboratories, there was no firm basis in 1957 upon which to predict the appearance of that dangerous complication of influenza, namely, influenza virus pneumonia.

SUMMARY

In human beings and in experimental animals, influenza virus causes a cytonecrosis in the ciliated respiratory epithelium. This results in an epithelial wound which facilitates secondary infection with respiratory pathogens, including the dangerous groups of pyogenic cocci.

During the pandemic of Asian influenza (1957–1958) many people died from a curious form of pneumonia without coexistent bacterial infection. The histopathology resembles closely some of the descriptions of fatal 1918 influenzal pneumonia. The same type of pneumonia was seen in the winter of 1960. Microscopic analysis of those cases shows that it is very probable that the virus attacks the alveolar cell lining, subsequently causing its destruction. The vascular damage is considered to be a phenomenon secondary to the destruction of this lining. Experimental influenza virus pneumonia in mice is basically the same process.

Unknown host factors favoring the development of influenza virus pneumonia most probably do exist. So far, it is not certain that the

Asian virus of 1957 has manifested an enhanced genetic human pneumotropic virulence.

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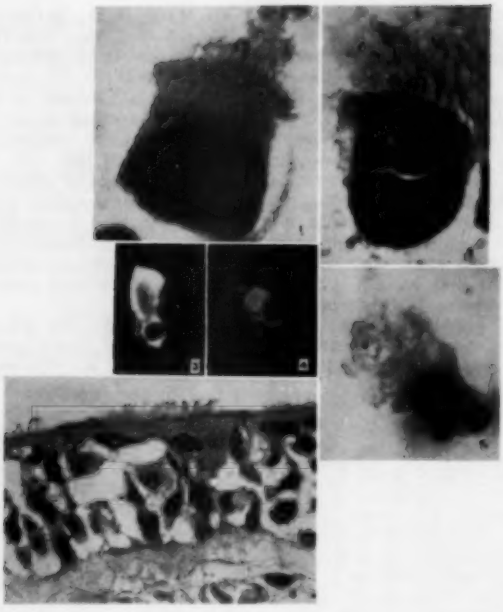
(*Figures on following pages*)



Patients:

Case 1. (F), aged 39 (1957). Fatal Asian (A2) influenza virus pneumonia, mitral stenosis and pregnancy, about third day of disease.

Case 2. (F), aged 45 (1957). Fatal Asian (A2) influenza virus pneumonia. Mitral stenosis, about third day of disease.

Case 3. (F), aged 39 (1960). Fatal A2 influenza virus pneumonia. Mitral stenosis and pregnancy, about third day of disease.

Case 4. (F), aged 62 (1960). Fatal A2 influenza virus pneumonia. Aortic valvular stenosis, about fourth day of disease.

Case 5. (M), aged 20 (1960). A2 influenza, third day of disease.

FIG. 1 (C). *Case 3.* Wett methylene blue preparation of smear from tracheal epithelium. Cytopathic change in ciliated cell, showing disappearance of tail and a swelling of the nucleus, which is situated transversely in the cytoplasma. Cilia visible. (Oil-em.)

FIG. 2 (C). *Case 3.* Wett methylene blue preparation of smear from tracheal epithelium. Cytopathic change in ciliated cell, showing phagocytosis of shrunken cell and a pyknotic nucleus. (Oil-em.)

FIG. 3 (C). *Case 3.* Smear from tracheal epithelium showing bright specific fluorescence in cytoplasm of ciliated cell, with small round nucleus. "Absorption" of erythrocytes on the cell border (Fl.). (Oil-em.)

FIG. 4 (C). *Case 3.* Smear from tracheal epithelium showing specific fluorescence in cytoplasm of degenerated ciliated cell (Fl.). (Oil-em.)

FIG. 5 (C). *Case 5.* Wett methylene blue preparation of nasal exudate. Cytopathic change of ciliated cell with nuclear fragmentation. (Oil-em.)

FIG. 6. Small bronchus (H). *Case 2.* Cytopathic changes in ciliated epithelium. Cilia partly preserved. The vacuoles might be intercellular spaces which may have developed as a consequence of the shrinkage of ciliated cells. In one of these spaces an erythrocyte is seen (H.P.T.). (Oil-em.)

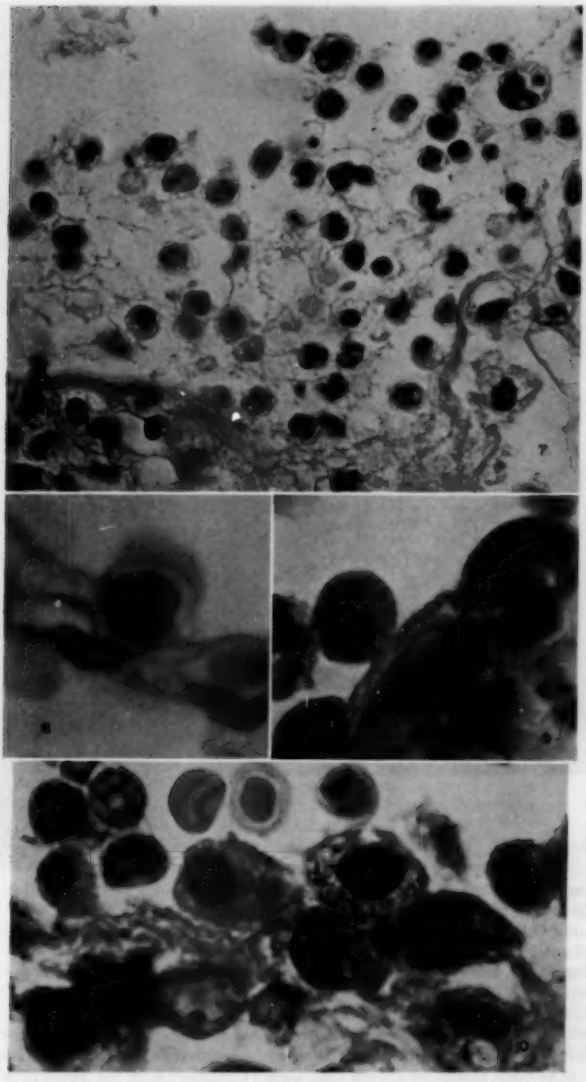


FIG. 7. Lung (H). *Case 1.* Exudate in alveolar duct, showing some fibrin, a few leukocytes and a few alveolar cells. Most cells are small, with round pyknotic nuclei, situated eccentrically in the cytoplasm. One histiocyte (macrophage) is seen showing phagocytosis of cellular debris (H.P.T.). (Low magnification.) It is interesting to compare this microphotograph with plate 60 of the publication of MacCallum in 1920 (20).

FIG. 8. Lung (H). *Case 2.* Alveolar septum, with attached normal alveolar cell (H.A.). (Oil-em.)

FIG. 9. Lung (H). *Case 2.* Alveolar septum with attached alveolar cell, showing rounding and a pyknotic nucleus, situated eccentrically in the cell (H.A.). (Oil-em.)

FIG. 10. Lung (H). *Case 2.* Alveolar septum with hyperplasia of alveolar epithelium. To the left 2 cells showing cytopathic changes (shrinkage of cytoplasm and pyknotic nuclei) (H.A.). (Oil-em.)

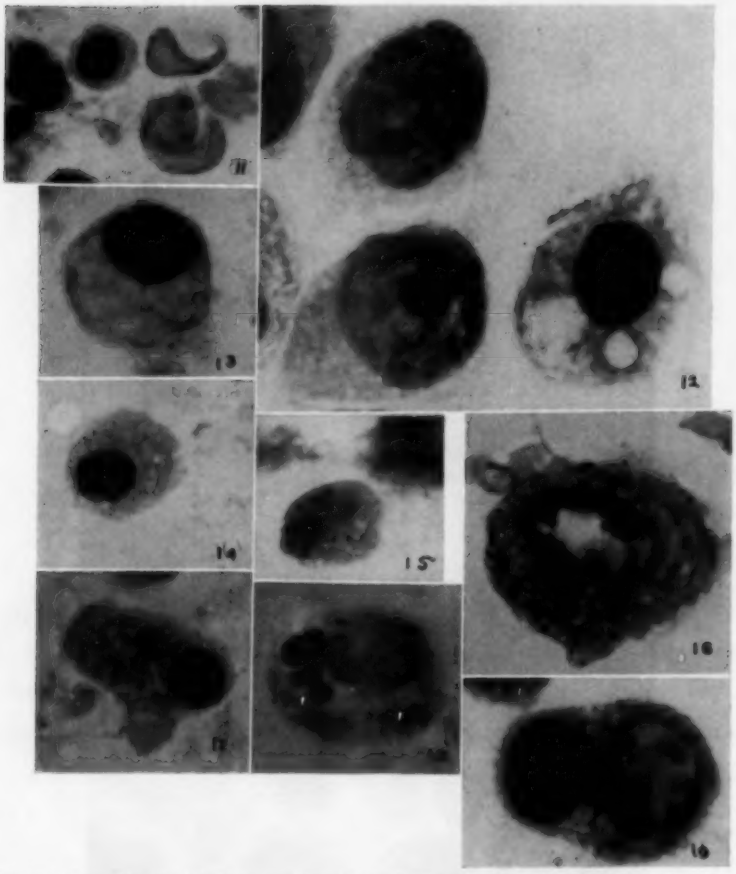


FIG. 11. Lung (H). *Case 2.* Very small, most probably alveolar cell, situated in the alveolus amidst erythrocytes, showing a pyknotic nucleus (H.A.). (Oil-em.)

FIG. 12. Lung (C). *Case 3.* Hyperplastic alveolar cells, and alveolar cell showing cytopathic changes (pyknotic nucleus; vacuolization) (H.E.). (Oil-em.)

FIG. 13. Lung (C). *Case 3.* Shrunken, probably alveolar, cell. Pyknosis of nucleus which is situated eccentrically in the cell and vacuolization (H.E.). (Oil-em.)

FIG. 14. Lung (C). *Case 4.* Shrunken, probably alveolar, cell. Small round nucleus with two fragments of chromatin situated at the nuclear membrane (H.E.). (Oil-em.)

FIG. 15. Lung (C). *Case 4.* Shrunken, probably alveolar, cell showing nuclear fragmentation (H.E.). (Oil-em.) It can be shown that nuclear fragments in the same type of cells contain deoxyribonucleic acid, using the bicolor method for deoxyribonucleic acid and polysaccharides (acid hydrolysis-thionine SO_2-PAS), according to van Duijn.

FIG. 16. Lung (H). *Case 3.* Normal alveolar histiocyte (macrophage) in alveolus (H.P.T.). (Oil-em.)

FIG. 17. Lung (H). *Case 3.* Cytopathic change in alveolar histiocyte (macrophage) showing small round pyknotic nucleus, situated eccentrically in the cytoplasm (H.P.T.). (Oil-em.)

FIG. 18. Lung (H). *Case 2.* Cytopathic change of alveolar histiocyte (macrophage) showing nuclear fragmentation (H.A.). (Oil-em.)

FIG. 19. (C). *Case 3.* Wett methylene blue preparation of smear from lung-tissue. Alveolar histiocyte (macrophage) showing phagocytosis of small cell with pyknotic nucleus, situated eccentrically in the cytoplasm. (Oil-em.)

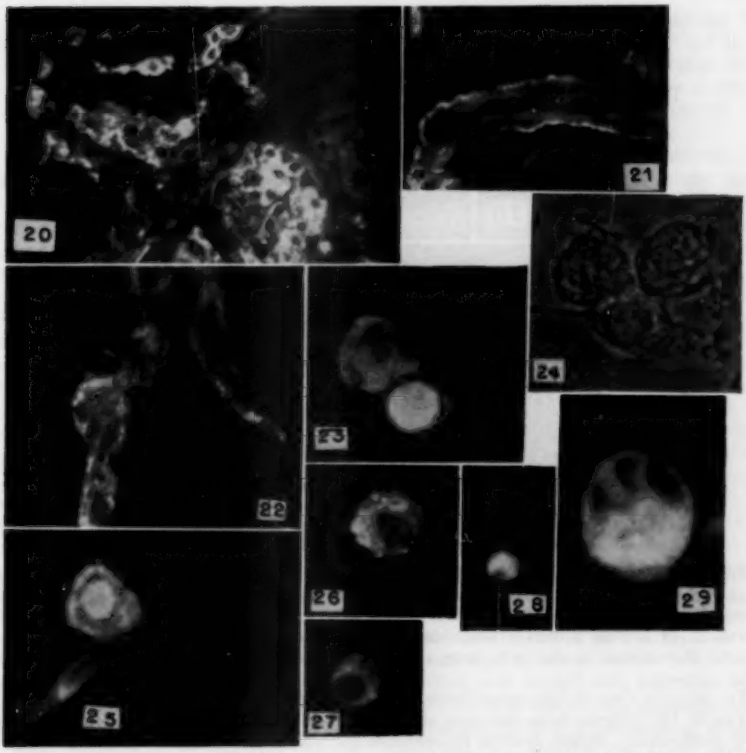


FIG. 20. Lung (H). *Case 3.* Hyperplastic alveolar epithelium lining alveolar septa and showing bright specific fluorescence (Fl.). (Low magnification)

FIG. 21. Lung (H). *Case 3.* Alveolar septum with bright specific fluorescence in cytoplasmic lining probably belonging to the alveolar epithelium (Fl.). (Oil-em.)

FIG. 22. Lung (H). *Case 3.* Specific fluorescence in alveolar cells and histiocytes (macrophages) covering the alveolar septa (Fl.). (Oil-em.)

FIG. 23. (C). *Case 4.* Smear from lung tissue showing 3 alveolar cells. One cell shows bright specific fluorescence in the nucleus; a second in the nucleus as well as in the cytoplasm. The third cell does not show fluorescence (Fl.). (Oil-em.)

FIG. 24. (C). *Case 4.* The same cells from figure 23 in phase-contrast. (Oil-em.)

FIG. 25. (C). *Case 4.* Smear from lung tissue. Alveolar cell with bright specific fluorescence in nucleus and in the periphery of the cytoplasm (Fl.). (Oil-em.) A few other cells show no fluorescence.

FIG. 26. (C). *Case 3.* Smear from lung tissue. Alveolar cell with specific fluorescence in the cytoplasm. Clubbing of cytoplasm. Nucleus, situated eccentrically in the cytoplasm. It lacks nuclear fluorescence (Fl.). (Oil-em.)

FIG. 27. (C). *Case 3.* Smear from lung tissue. Small, most probably alveolar, cell with small round nucleus situated eccentrically in the cytoplasm. Specific fluorescence in the cytoplasm (Fl.). (Oil-em.)

FIG. 28. (C). *Case 3.* Smear from lung tissue. Very small, probably alveolar, cell with bright specific fluorescence. Possibly very small nucleus or nuclear fragment at cell border (Fl.). (Oil-em.)

FIG. 29. (C). *Case 4.* Smear from lung tissue. Alveolar histiocyte (macrophage) with bright specific fluorescence in cytoplasm. Nuclear fluorescence is lacking (Fl.). (Oil-em.)

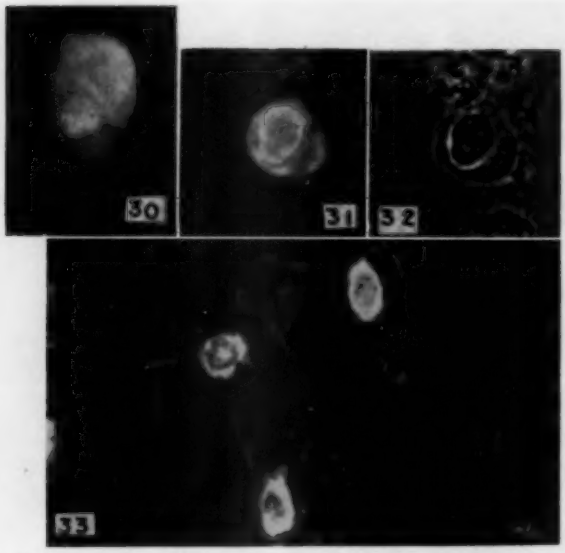


FIG. 30. (C). *Case 4.* Smear from lung tissue. Alveolar histiocyte (macrophage) showing a bright fluorescence in the nucleus and a bright fluorescence in a phagocytosed small, probably alveolar, cell (Fl.). (Oil-em.)

FIG. 31. (C). *Case 3.* Smear from lung tissue. Alveolar histiocyte (macrophage) showing specific fluorescence in the cytoplasm and in a phagocytosed small, probably alveolar, cell (Fl.). (Oil-em.)

FIG. 32. (C). *Case 3.* The same histiocyte from figure 31 in phase contrast. (Oil-em.)

FIG. 33. (H). Mouse lung, two days after intranasal infection with swine influenza virus A (10^{-2} hem.-units). Bright specific fluorescence in cytoplasm of 3 alveolar cells, lining the alveolar septa. Two cells show nuclear fluorescence as well. Fluorescence of cytoplasmatic lining of the alveolar septa is seen between the cells (Fl.). (Oil-em.)

The microphotographs were taken from histologic sections (H) and cytological smears (C) from lungs, trachen, and nasal exudate of 4 virologically confirmed fatal cases and one clinical case of Asian (A2) influenza, without coexistent bacterial infection (1957 and 1960). Alveolar (septal) epithelial cells are indicated as alveolar cells, in contradistinction to alveolar histiocytes or macrophages. Zeiss photomicroscope was used for light microphotographs. Film: Ilford Pan F. Oil-em. (100 ×) and low power (40 ×).

Staining methods and fluorescein-labeled antibody technique:

H.A. = Hematoxylin-azophloxin.

H.E. = Hematoxylin-eosin.

H.P.T. = Hematoxylin-phloxin-tartrazin (according to Lendrum).

Fl. = Fluorescein-labeled antibody technique, using the indirect method ("sandwich") according to Coons, modified by Goldwasser and Shepard and based on the principle of a complement-fixation test. Human convalescent antiserum from cases of A2 influenza was used. The conjugate, a rabbit anti-guinea pig gamma globulin, labeled with iso-thyocyanate, was kindly supplied by Dr. D. A. J. Tyrrell and Major L. L. H. Kent. Uninfected lung tissue of human beings treated with specific antiserum, and infected lung tissue treated with normal ferret serum served as controls. Unfixed tissue blocks were freeze-dried in vacuum at −45°C. for three days. Vacuum embedding in paraffin wax (melting point 50°C.) was done in ten minutes.

Microscope: Zeiss fluorescent equipment. Light source: Osram H.B.O. 200. Exiter filter B.G. 12. Barrier filter G.G. 4 and O.G. 4. Film: Super Ansco chrome artificial light. Exposure time: 40 seconds (Oil-em., 100 ×), and 10 seconds (low power, 40 ×).

DR. C. H. STUART-HARRIS: To discuss Professor Mulder's paper in terms other than admiration would, I think, be a matter of effrontery. I for one have greatly enjoyed this demonstration of the virus lesion in man which has so many parallels, not only with human pathology in 1918, but with the experimental lesion as it is known in experimental animals. Professor Mulder very kindly sent me a manuscript of his paper before I came over, and I believe it would be better if I concentrated on some other aspects of the pathology in man.

Therefore, I am going to review for you the findings which have been made by Drs. Hoult and Flewett during their studies of the cases they described recently in *The British Medical Journal* (1960, *1*, 1847) of fatal encephalitis following influenza. They have described six cases in all. One was a case of post-influenzal variety of demyelinating disease. In ordinary preparations ring hemorrhages are seen; with myelin staining the ordinary pattern of demyelination is demonstrated. An unusual feature, perhaps, is that demyelination does not extend very far from the actual vascular structure in the middle of the lesion. Among the other five patients, there were lesions that were found only on very careful search. One which was found in each of four patients showed small areas of infiltration along vessels leading from the meninges. There was a marked degree of meningeal reaction, and these areas were found in many parts of meninges covering the brain, cerebellum, or cerebrum. In the cerebellum proper, a number of lesions were found only after very careful search. I am not an anatomist, and it would be quite an impertinence for me to say that this is a typical lesion of viral encephalitis. However, I understand that this is the sort of lesion which is acceptable to pathologists and which is found in some of the known viral encephalitides.

High-power views of brain lesions show an infiltration with microglial cells spreading out from the central area of the cerebellum. The pathologic changes in the cells in areas where there has been infiltration are seen in the nuclear membranes with folding and fragmentation of the latter. I suppose it is possible—and the authors suggest that this is so—that changes in the Purkinje cells could represent some sort of reaction to the deeper infiltrative changes and are not necessarily due to primary cell damage. Changes seen in the granular layer in the cerebellum with a fallout of cells is again extremely suggestive of some sort of specific process. Microglial cell infiltration was seen in the cervical spinal cord in one of their patients. Well, as I said before, I think these histologic changes in the brain and meninges establish a prima facie case for bringing back the old concept that influenza at times causes a true encephalitis and that these changes are not necessarily of the postinfection variety which follows any acute infective process.

DR. ROBERT OSEASOHN (Cleveland, Ohio): I would like to summarize very briefly one particular aspect—that of extrapulmonary virus localization—in our experience with 33 influenza-associated deaths which occurred in Cleveland in 1957 (Oseasohn, R., *et al.*: New Engl J Med, 1959, *260*, 509). You may recall that in 1956, Dr. Loosli and co-workers described viremia and the presence of virus in nonrespiratory sites in experimental influenzal infection in animals. It was, therefore, of particular interest to us to see if it were possible to observe similar phenomena in the course of fulminating fatal influenza in man. Of our 33 influenza-associated deaths, Asian strain virus was isolated from the respiratory tract, that is, lung or trachea, in 25 of the cases. It was possible to obtain tissues from extrarespiratory sites in 15 of these respiratory tract-positive cases. From 3 of them, influenza virus was isolated from extrapulmonary tissues.

As you have heard today, one particular bugaboo in a study of this kind is the matter of contamination of specimens taken from viscera other than lung and trachea with secretions from these latter organs. It was for this particular reason that we observed extreme precautions in obtaining our specimens. As soon as the body cavities were opened, using washed, flamed instruments, we removed all of the samples that we will discuss. The details of the techniques have been published in the *Proceedings of the Society for Experimental Biology and Medicine*, 1959, *100*, 272.

I would like to review some of the clinical, anatomic, and laboratory findings in the 3 cases from which virus was isolated from extrapulmonary tissues. The ages of these patients were ten, fifty-nine, and four years. The duration of illness was between two and four days. Of particular interest is the observation that both children gave no clinical history, nor was there any anatomic evidence, of pre-existing disease. The one adult patient, a fifty-nine-year-old female, had been hospitalized and studied repeatedly because of liver cirrhosis, presumably due to alcoholism. The bacteriologic findings are of interest with reference to what has been said earlier about the staphylococci. Hemolytic staphylococci were isolated from the lung and trachea in the ten-year-old. Cultures of the lung and trachea of the second patient, the cirrhotic, not treated with antimicrobials, were sterile. The four-year-old girl had been treated by her par-

ents with penicillin tablets left over from a preceding illness. Her lung was sterile, but hemolytic staphylococci were isolated from the trachea.

The gross appearance of the respiratory organs in the first case—that of the ten-year-old boy—showed an acute hemorrhagic and necrotic tracheobronchitis. His lungs were bulky, airless, weighed several times more than normal, and were characteristically dark blue-violet in color.

Cut sections of the lungs showed areas of confluent lobular pneumonia scattered throughout both lungs. In contrast to what has been said earlier, on cutting the lungs, fluid literally gushed forth. They were extremely wet. The chief characteristics of the lungs, to which we would like to draw attention, were the extensive hemorrhagic changes, the pulmonary edema, and the scattered changes throughout both lungs.

Now what about the histologic appearance of the viscera, exclusive of the respiratory tract, from which influenza virus was isolated? Of the 15 cases that were respiratory tract-positive for virus, virus was isolated from the viscera in 3 cases. In the first patient (the ten-year-old boy) virus was isolated from spleen and lymph node; in the next patient (the fifty-nine-year-old cirrhotic), from liver, spleen, kidney, and heart; and in the last patient (the four-year-old girl), from the tonsil. In the first case the spleen and liver showed a nonspecific inflammatory response, while in the second there was evidence of preexisting disease such as portal cirrhosis, nephrosclerosis, and chronic pyelonephritis. Of particular interest were the changes in the arterioles of the myocardium of the fifty-nine-year-old female. They showed fibrinoid degeneration with focal fibrin thrombi. It is of interest that of 13 samples of myocardium that were examined, those from 3 individuals exhibited histologic evidence of myocarditis. From none of the myocardium samples of the 3 was virus isolated. With reference to what has been said earlier about the involvement of the oropharynx, it is of interest to point out that the four-year-old child showed a marked fulminating acute follicular tonsillitis, with frank necrosis of follicles.

With reference to the matter of assay of the tissues for the content of virus, cases were included as examples of isolation of virus from extrapulmonary sites when it was possible to repeat the isolation of virus on three consecutive attempts from the original material. Virus was then assayed in the 3 cases to which reference has been made. Lung material was diluted from 1:10 to 1:10,000,000 and the extrapulmonary tissues, from 1:5 to 1:500. The concentration of virus in tissues from these cases was determined. This concentration was defined as the reciprocal of the highest dilution of suspension from which virus was isolated after the first amniotic passage. In the respiratory tract tissues, the concentration of virus ranged from 100 to 100,000 and in the extrapulmonary tissues, from 5 to 1,000.

We have interpreted these findings to show that there is certainly a good reason to believe that viremia does occur, perhaps as a "spill-over" phenomenon, in fulminating fatal influenza in man. However, we made no attempt in these studies to isolate virus from the blood.

Dr. Howard T. Karsner (Washington, D. C.): When Dr. Loosli put me on the program, I wondered why, because I have had no experience whatsoever with Asian influenza. I made some inquiries and I was given to understand that the reason was that I am one of the few men in the world who went through and survived the outbreak of Spanish influenza.

Certainly to me this whole program has been most instructive. I want to point out, however, two little things. My only experience was largely that of post-mortem examination of a large number of people who died in Cleveland at the Lakeside Hospital, and in what was then called the Cleveland City Hospital. But, being naturally somewhat curious, I did inquire about the matter of clinical manifestations. What I have to say is largely based on memory, and memory, as everyone knows, is fallacious. I have put down some of my items, and I have asked two of my former associates of Cleveland, Dr. Koletsky and Dr. Kinney, to verify these as far as possible.

In the first place, in that outbreak, there was no such extensive clinical study of patients as has been demonstrated here today. There was no battery of various tests. The mere fact that a patient was sick did not mean that an electrocardiograph was brought in at once. Many of the functional studies which have been reported at this conference were not done in those days. Certainly we knew nothing about the virus, and the idea of separating viral disease from bacterial disease was utterly impossible. What we saw then was largely the result of a combination of viral disease and particularly bacterial disease. Referring to Dr. Stuart-Harris' comments about the central nervous system, I would say that my recollection, which has been verified, is to the effect that no observations were made to justify intensive study on the central nervous system. Nor did we see anything to indicate that there was myocardial disease. Another thing that has developed since then is a careful bacteriologic study of the living patient and of the dead tissues. In those old days, although bacteriologic studies were made, they were precise and exact, if not true. My recollection is that, instead of finding

staphylococci, we found innumerable streptococci. I think that may have been due to the fact that, when we found staphylococci in those ignorant days, we looked on them as contaminants, so that what we had to say about bacterial contamination was not valid.

One of the things that impressed me greatly, from the clinical aspect, was the number of instances in which people died abruptly after two or three days' illness of what appeared to be cardiovascular collapse—medical shock, if you want to call it such. At the post-mortem examination of these people, we did find dilatation of the heart and a great deal of edema of the lungs, but in going over the sections again we have not been able to find any clear-cut evidence of myocarditis. Some of you have heard of the banjo player who played the banjo better than his teacher did, and I confess that Dr. Saphir has far greater knowledge of myocarditis than I would ever pretend to have. However, I think even in those days we were not so stupid that we would fail to see outspoken myocarditis. That there may have been focal myocarditis is certainly true, because our examinations of the heart were not so extensive as present examinations.

I have been impressed by the emphasis placed on the necrotizing tracheitis, tracheobronchitis, and even bronchiolitis. I was attuned to that sort of thing because I had a great deal of experience in World War I with diseases of the trachea and bronchi in connection with mustard-gas poisoning, so I don't think that I was so stupid as to overlook this altogether. However, we found it in only 2 cases. Another thing that has impressed me greatly is the demonstration in the beautiful illustrations of lung changes of what is now called, or what is often called, hyaline membranes. We found hyaline membranes in only 2 cases.

That may be a reflection of the fact that in our cases we did not see this profound disease of the tracheobronchial tree. We did not isolate *Hemophilus influenzae*; I think we were too stupid to do so. We did not find anything that would suggest to us that we were dealing with anything other than a profound and mixed pneumonia. The disease of the lungs was most severe and was most impressive. Dr. Oseasohn has shown you some of the sections of lungs. We could have duplicated those sections over and over again had we had the facilities to take color photographs. But the lungs of our patients—and the patients varied a great deal in age—showed the most profound and varied disease, anywhere from hyperemia to hemorrhage to exudate of different kinds in different parts of the same lung. We said in those good old days that this patient had bronchopneumonia, lobar pneumonia, hemorrhagic pneumonia, and so forth and so on, all present in the same lung. That we were dealing, at any time, with what Drs. Stuart-Harris and Mulder have referred to as influenzal pneumonia is extremely doubtful.

Dr. Maxwell Finland (Boston, Massachusetts): I should probably not have gotten up at all, particularly after the beautiful demonstrations of the pathology of the lungs which Professor Mulder and Dr. Oseasohn have shown. However, I thought it would be interesting to show some slides of the microscopic pathology of uncomplicated influenza viral pneumonia as observed in different years at the Boston City Hospital (*slides not reproduced here*).

The first set of 3 slides are from the collection of the late Dr. Frank B. Mallory and are from a patient who died during the pandemic of 1918. One of them shows a dilated alveolar duct lined with a dense hyaline membrane; the second shows alveoli filled with edema fluid and an exudate of large mononuclear cells, and the third shows a bronchiole with intact epithelium, mucus and mononuclear cells within the lumen, and the characteristic edema in the surrounding alveoli. This is one of many cases from that epidemic in which Dr. Frederic Parker, Jr. and Miss Marion E. Lamb made intensive studies for bacterial pathogens; they found none in this case by culture, and Dr. Mallory failed to demonstrate any after intensive search in histologic sections.

The second set of slides looks like an exact duplicate of the first; the slides are from a similar case of pure influenza A viral pneumonia that occurred in January, 1944 (Amer J Path, 1946, *22*, 797). The identical picture was also seen at this hospital in a pure influenza A1 viral pneumonia complicated by a myocardial infarct in 1953 (Amer J Hyg, 1953, *62*, 1) and also in cases of Asian influenza (AMA Arch Intern Med, 1959, *103*, 515).

The identical pathologic picture in influenza viral pneumonia due to influenza viruses A, A1, and A2 is in line with the discussion which Professor Stuart-Harris started earlier today. I might also have shown you sections from cases of staphylococcal pneumonia complicating influenza A occurring in January, 1940 (Amer J Path, 1943, *19*, 23) and Asian influenza in 1957 (AMA Arch Intern Med, 1959, *103*, 515) which are also indistinguishable.

INFLUENZA IN PERSPECTIVE[1]

THOMAS FRANCIS, JR.

Introduction

After a long day of fever, fun, and food, it is somewhat foolhardy to talk more about "flu." And I'm led to ask, "How did I get into this situation?" As most of you appear to be comfortably seated my assignment might be construed to indicate that, as you relax there, I should attempt an "analysis" of our psychic and scientific status with respect to influenza. But there are great risks in such an undertaking. There is the risk that one may miss certain significant perspectives because of myopia, or because of cataracts which come from too constant exposure to the flame. I am reminded of Erskine Caldwell's shareeropper who, when the pressures got too great, would go into his shed with a jug of corn liquor and relax by fixing his eye to a knothole in the wall and just look. That is certainly an unbiased approach.

I can only undertake to look at a few aspects of the field of influenza and to discuss the view seen from one man's knothole—with the use of as little corn as possible. I shall, therefore, review some phases of progress from the vantage point of the 1957 epidemic and its present recurrence to see what we learned and what future needs were emphasized. The thirty years during which out technical knowledge of influenza viruses has been acquired is a brief period in the centuries of influenza's history, and it is impossible to talk of the disease, influenza, without the perspective of earlier history, for so many of the phenomena we seek to explain are founded in pre-viral records.

Little has been added to the basic clinical description of Short (1557) 400 years before the Asian epidemic. The essential epidemiologic characteristics were repeatedly described in the reports of several centuries. Contagiousness was accepted. Then, as now, discussion centered on the origins of the outbreaks; on the mode of spread; on the rapid dissemination; on the age distribution; on the fatal effects among the very young, the old, the infirm, or those with unsound viscera; on concomitant illnesses in animals although swine are not mentioned; on the influenza *di freddo* or the winter factor. But it was recognized that large outbreaks were not limited to winter.

In certain "pandemics" of the eighteenth century the frequency of complications was emphasized and the increased risk to males of sixteen to forty-five years of age was reported. The pandemic of 1743 may have been as severe proportionately as that of 1918. The descriptions are so consistent and in keeping with current virus-confirmed observations that there can be little doubt that these epidemics were usually influenza. Hecker, however, complains that the leading physicians of the sixteenth century at the time of the sweating sickness "occupied themselves rather with art than with actual observation, and in their critical researches overlooked the important events that were passing before their eyes." Obviously, this was not wholly true.

These thirty years of influenza virus research may be looked upon as the period of discovery, exploration and explanation, and effort to map the general ecology of influenza viruses. Hypotheses, speculations, and trial balloons, or "fluniks" have been abundant, and some of them have been valuable. In these thirty years, firm strongholds of scientific knowledge have been established which serve as bases for further advance. Methods of specific diagnosis, clinically and epidemiologically applicable, have permitted increasing accuracy in separating influenza from the complexity of respiratory illness. Nor should one overlook the influence of influenza research on the development of research into the rest of the respiratory field, or on virus research generally.

The role of serologic variation in epidemic recurrences has been demonstrated. The pandemic distribution of a virus strain has been repeatedly observed. The spread of several epidemics from northern to southern, from eastern to western hemispheres (or vice versa) has been charted. The occurrence of repeated, apparently localized flurries of a virus has been repeatedly noted well in advance of a full-blown epidemic prevalence, indicating that the establishment of multiple foci is a significant forerunner and that the pandemic distribution is not

[1] A critique presented in an informal manner at the evening session on February 17, 1960.

a spontaneous eruption of the same variant in all parts of the world simultaneously. One of the most important observations is that a sharp serologic variation of itself is not indicative of high virus virulence and high case fatality. No sudden accession of virulence during proved viral epidemic or pandemic prevalences has been noted.

From a limited series of interested listening posts established informally in the United States, an extensive chain of laboratories has developed throughout the world, collaborating in the detection and study of influenza.

There has been a tremendous advance in immunologic knowledge and repeated demonstrations that vaccination can give a high degree of protection against experimental or natural disease, and that it is generally applicable. But because it requires energy and expense we witness the querulous complaints that reactions and uncertainties of the future are too great. This is unscientific squeamishness often used to cover up inertia.

Recognition of the specific cytopathic effect of influenza virus on the respiratory epithelium of man and animals and its similarity to the histologic pathology of the 1918 epidemic emphasizes the similarity in pathogenic agents involved. The role of bacteria in pathogenesis has been more clearly visualized, especially in the severe forms of the disease. The elegant studies of Hers and Mulder and associates, especially, have provided the picture of apparently pure virus pulmonary pathology and the altered, almost specific, characteristics in the presence of certain bacterial invaders. The lethal role of the Staphylococcus has been increasingly established, and re-study of the 1918 sections has shown its participation in fatal pneumonias of that period.

Virus Investigations

A tremendous amount of study has centered upon the virus, as organism and as reagent. This is to be expected since laboratories tend to study the products of their cultivation, and an agent so readily handled with a great variety of techniques and with low risk to personnel becomes a tool for multiple types of investigation. I am reminded of pneumococcus days when so many articles began with the phrase, "In 1917 Dochez and Avery...." Now it is, "In 1933 Smith, Andrewes and Laidlaw...." The contributions of the microbiologic laboratory added much basic information on the pneumococcus, its antigenic analysis and virulence, as well as fundamental knowledge of great general biologic importance, especially to genetics. But it was a long way from providing understanding of the origins, the pathogenesis, and the pathophysiology of lobar pneumonia. These interests were greatly dampened with the appearance of chemotherapy.

This conference, perhaps wisely, has chosen to emphasize the disease problem rather than the virus. I would, nevertheless, like to comment in a general way on some features of the viral investigations.

Immunologic Relations

Among the earliest serologic studies of swine and human influenza viruses were those demonstrating the clear differences between them. Nevertheless, animals that recovered from infection with one exhibited significant resistance to the other; and hyperimmunization of ferrets, mice, or swine with one elicited antibodies to the other. The ability of A1 and Asian strains to induce cross immunity to earlier strains has also been demonstrated by hyperimmunization. Recognition of the variation among human influenza viruses led almost to a stampede in the demonstration of differences, although the boundaries of significance in these variations have been difficult to establish. A variety of classifications promptly erupted—old world—new world—races—types. Dr. Magill and I thought in 1938 that these divisions were scarcely justifiable in view of the great crossing. When the identification of type B strains occurred, a better basis for organization seemed apparent. But then the debate on binomial classifications—and the lovely girl, Tarpea—occurred. And now we have parainfluenza virus—which offends because it is a weasel word. Influenza is a disease with clinical and epidemiologic characteristics providing names for the influenza viruses, not an *in vitro* designation for something which may have nothing to do with influenza.

There can be little doubt that any given strain of a type will possess (apart from the type-specific antigen), a series of antigenic components relating it to other strains. Those from an epidemic season have great similarity. After the apearance of a major antigenic variant, strains of the next several years may show some moderate serologic deviations, but they appear to be random alterations or drift, as Burnet calls it,

rather than an orderly chronologic or directional progression. Thus, the examination of strain antigenicity together with epidemiologic data tends to establish periods of prevalence of major variants, in keeping with the older concept that a large outbreak and its trailers constitute a pandemic period, limited perhaps by saturation of the population. The associated strains tend, then, to comprise a group or family within the type. Vaccination with one member of the group appears to induce effective immunity against other members.

On the basis of the early studies on cross immunity, I had supported the hypothesis that the presence in man of antibodies against swine influenza represented only the effect of overlapping common antigens in the human strains. This view has been modified by the later data demonstrating rather specific characterization of age groups by their dominant strain-group antibody, which corresponds to the virus group prevalent in the childhoods of the respective cohorts. In this respect it is noteworthy that the age pattern of antibody to the major variants is quite similar in widely separate parts of the world. The age at which antibody to swine and PR8 strains is first demonstrable has been observed to advance with the passage of time in a surprisingly accurate fashion. Hence, one does not have antibody in high titer against each and every strain simply by compounded experience but distinctively, depending upon the strain of initial infection. Absorption of the serum of a given age group with antigen corresponding to its dominant primary antibody removes not only that antibody but the antibody to all other groups as well. Repeated experience plays an important role in enhancing the characteristic group antibody, as can be shown by vaccination or reinfection experiments. These observations have been valuable guides in the formulation of a perspective regarding population immunity and preventive vaccination.

Another fundamental contribution of the viral studies has grown from the basic observations of hemagglutination and the character of the virus-cell union. The similarity to an enzymic reaction was promptly recognized by Hirst; the similarity between virus absorption and elution of virus from respiratory tract tissues and that with erythrocytes was pointed out, and study of the nature of the cellular substrate was begun. Investigation of the reaction has been carried to advanced conclusions at the biochemical level with elegant demonstrations of sialidase and neuraminadase. But, further, the work has led to the concept of different cell receptors corresponding to serologic variation, of the process by which cellular infection is initiated and, in effect, toward defining the mechanisms of the cytopathic and pathogenic effects observed in actual disease of animals and man. These developments provide an intriguing base for consideration of therapy and preventive measures.

This brings up the subject of inhibitors. Although it was recognized early in mouse neutralization tests that the serum of some normal animals, such as rabbits, contained a low degree of neutralizing effect against some strains, the use of the hemagglutination-inhibition test has revealed a complexity of inhibitors in various sera and other materials. Some are heat stable, some are enhanced by heat, some are heat labile. Many relate to muco- or lipoproteins. Their significance in natural or physiologic resistance is not established, and this field needs more systematic study.

Attention turns, therefore, to the viral factors involved. The varying behavior of strains with one or other inhibitor is recognized. How much of this is dependent upon the medium employed for propagation and how much is a native characteristic is at times uncertain since two lines of the same strain isolated and maintained in different systems may differ considerably. We have been led to the suggestion that the increased susceptibility of new strains to the heat-stable serum inhibitor may be an index of their epidemic potential, indicating that they have an increased ability to combine with mucoid receptors in the body tissues and thus increase their infectivity.

A reciprocal development has been the so-called "phase variation" in strains, based upon the reactivity of the virus with homologous and heterologous antibody. These effects appear to be related to the amount of the virus coat and matrix which may alter the surface availability of the constituent antigens; their behavior can also be influenced by propagation in different systems.

The developing work on the structure and composition of influenza and similar viruses is providing great stimulus to exploration. The isolation of the nucleoprotein as the type-specific

antigen and genetic director of the virus, of the hemagglutinin as a mucoprotein with a variety of activities including that concerned with specific immunity, and of the mucoprotein coat presumably derived from the cell wall gives a simplified working base for study. Combined with electron microscopic studies of virus embryology, anatomy and malformations, concepts of virus organization, maturation and hatching are taking form. In this connection, demonstration of the effects and site of effects of large active or inactive inocula vis-à-vis smaller amounts teases the imagination. Interference, interferon, interfera—from the vulgate. This phenomenon is widely considered at present. Although it has not reached the point of a practical test, it is a very provocative product.

The growth of biochemical and biophysical knowledge provides further basis for the preparation of purified and compounded antigens. While current information on the genetics of influenza variation or mutation seems inadequate for generalization on viral genetics, the studies of recombination and induction may also lead to practical application. Intriguing studies of cell metabolism and cell-virus interactions *in vitro* offer continued promise of major contributions to possible chemotherapy or prophylaxis.

This, in general, was the state of knowledge as the 1957 pandemic developed. Great advances had taken place, much important information had been acquired; but how much assistance did this provide toward meeting the impending storm? There were several lines of acquired perspective of practical value, including the epidemic behavior of virus variants, experience with vaccination, the bacterial risks in the face of antimicrobial therapy.

At times it is said that interest in influenza would be slight, were it not for the specter of 1918. That doesn't hold, however, with the true "influenzologists," as someone called them. Influenza with its rapid large epidemics and increased mortality, its consistently capricious nature and its prestidigitating viruses constitutes an important and tantalizing problem under any conditions. Even in mild form it can cause a great disruption of community life and health. It is true, however, that the advent of any extensive outbreak conjures up the possibilities of a devastating epidemic with which we would not be able to cope. When, as in 1957, the appearance is associated with a significant immunologic variant, great publicity, and a wealth of investigators anxious to justify their interests, excitement rapidly mounts.

Concern was promptly raised that we should encounter a severe second wave as in 1918. The knowledge acquired during the previous virus studies did not support that likelihood. This was not the first extensive pandemic since virus discovery. That of 1936–1937 was identified throughout America, Europe, Asia, and Australia in a short time, even though a limited number of laboratories was in operation. It represented the highest excess mortality encountered in the United States after 1928. In 1943 the incidence was the highest after 1918. In 1947 a sharp serologic variant became widespread. In none of these was enhanced virulence exhibited as the epidemic progressed.

Although in adaptation of virus to experimental animals distinct enhancement of virulence is recognized, it seems that adaptation of virus to man is well advanced by the time it has become widely disseminated. Nevertheless, differences in pneumotropic capacity for ferrets and mice have been noted even in strains from the same year. It is apparent that antigenic variation can be an asset toward epidemicity of a strain, but is not the determinant of virulence. Consequently, the probabilities were high that it would not occur in 1957, but we still kept an eye on the mortality barometer. Besides, I have some uncertainty as to the nature of the so-called second wave of 1918.

The age distribution indicates that it was not a virus of entirely new antigenic structure; it seems, without doubt, to have been a "hot" one. But even then, some military units were heavily involved without fatalities. It was not winter—but late summer and an early pleasant autumn —when this alteration in severity of disease was recognized. I am not sure that the virus of the autumn was the same antigenically as that of the spring; while many observations of presumed cross immunity are cited, many others suggest there was none. If one accepts the Shope story, it was not until fall that the disease appeared in swine, which he attributes to the collaboration with Hemophilus. The sudden accession of virulence ascribed to virus of the second wave is not in keeping with knowledge of other epidemics of influenza—or other diseases—once well started. Were the bacterial agents the deciding factors? I don't know, but I don't think so.

Somehow I have a lurking suspicion that it was different virus from that of the spring. But I doubt that it was a recurrence of a fixed strain of some long past era.

There were some instances of a second wave in 1957, but generally it was more like a further extension and of the same character as the first spread except as mortality might be increased with winter weather. It appears that those affected in the later periods were different people. The second wave in reality is occurring now[2] with the probability again that different segments of the population might be involved. Its incidence in many areas is probably as high—the mortality as great or greater—but the excitement is reduced because of less publicity. It is impressive that so far this year there has been no major prevalence in the military population, which has been uniformly vaccinated.

What, then, did we learn from the 1957 pandemic? Frankly, little new was learned about the disease and its distribution. Many of its traditional features were reasserted and emphasized under the watchful eyes of prepared observers. It was the kind of developing storm that Kipling would have enjoyed reporting as "the storm came up like thunder out of China, cross the bay." There was high morbidity and low mortality. The initial point-to-point dispersions in many places of the temperate zone during the summer, according to the published accounts, were also observed in hotter climates such as India, and proceeded in the hot season to epidemic distributions of proportion. Menon's reported case total was only about 1 per cent of the Indian population and, if this is so (which I doubt), then the heat had a suppressive effect compared with the incidence of 25 per cent or more encountered during the main periods in other regions. But the winter season in the southern hemisphere also witnessed delays in epidemic build up. And I concur in the view that in 1957 the strain was a relatively poor spreader, requiring crowded groups or populations to build a good incidence. What might India and other overcrowded regions have had in winter or with a more expeditious summer traveler?

One of the most consistent features in all regions was the age distribution. Emphasis is placed on the major incidence between six and fifteen years of age. Influenza is always an important item in "The Child's Garden of Viruses." Next was the steady decline in incidence after twenty-five years of age, and there are indications of higher frequency of inapparent infection in old people. These indicate resistance of the older age groups. The presence of specific antibodies to Asian virus in old people provides some explanation, but I am impressed that a good proportion of persons from thirty-five to forty years on gave a secondary response to vaccination with Asian virus, indicating previous experience with the dominant antigen. Moreover, nearly all antibody against other type A groups as well as Asian antibody are removed from the serum of these elderly persons by adsorption with the Asian strain. We had observed earlier another antibody hump at fifty to seventy years of age in tests made with certain of the broad reacting A1 strains and sera of patients of all ages, and suspected it referred to a strain variant some years prior to 1918. We had even brashly postulated the appearance of an epidemic strain containing an earlier dominant antigen coming in through the immunologically open back door. It happened. This obviously is not a loss, never to recur, of antigens, as Andrewes had predicted.

Is this a rearrangement of antigens in the type A influenza virus under the pressure of population resistance, or is it a resurgence of a fixed strain which has been lingering for seventy years in an unrecognized reservoir? The latter view has been advanced on the basis that the 1889–1890 pandemic and that of 1957 arose in China; that the Asian strains are not neutralized by the beta inhibitor of mouse or swine serum, and that they agglutinate erythrocytes of horses and calves, as does the equine strain of Sovinova, hence predicating a swine or veterinary origin. It is said that some strains were recovered from swine in China, but the widespread serologic tests of animal sera have yielded no significant evidence of antibodies to the Asian strains. At the moment, then, there is no valid information of an extra human reservoir.

Interpretation of agglutinating activity and reaction to varied inhibitors is difficult to relate to epidemiologic characteristics. To illustrate: we have observed that heating of immune ferret serum reduces its hemagglutination-inhibition activity and can be restored by fresh guinea pig but not fresh mouse serum, a feature not previously noted. Should we assume that the

[2] Early in 1960.

guinea pig is an additional host in the infection chain? When normal swine serum is boiled, a tremendous increase in HI occurs versus Asian virus; is this the basis of a summer factor which reduces epidemics in hot weather? It is not effective against swine virus. Asian virus is not fixed by lung worm extracts but swine virus is, indicating that, in the event of a swine reservoir, the lung worm does not participate in the Asian plan. The receptors of Asian and swine virus on human erythrocytes are different.

Furthermore, I would like to point out that historically the swine relationship may have begun much earlier, for in Matthew 8:29-34 the casting of devils out of man into swine is described. This was obviously influenza.

My purpose is not to deride or condemn study of an influenza reservoir, but to emphasize that the meaning of the varied inhibitors with respect to virus characteristics is extremely vague. At present it is little more than serologic embroidery. I have already mentioned the idea that highly epidemic strains may, because of their great avidity for mucins, be especially susceptible to inhibitors of that nature. May it also be that the influence of cold weather is associated with the increased respiratory mucous secretions of that season, increasing the ease of spread and the exaggerated effect on persons with chronic cardiac or pulmonary disease. It may be similar to the effect of ether anesthetic in increasing susceptibility of animal hosts.

Our hypothesis has been that influenza virus of a given type contains a limited number of antigens which may be rearranged by chance, but the opportunity for widespread distribution is selective, depending largely on the pattern of population immunity and the chances of ready transfer. According to this theme, the great gap in immunity to the antigens of 1889–1890 permits the resurgence of that antigen to a dominant position, thus establishing a variant which could be disseminated through a highly susceptible population.

The 1957 studies also added valuable information regarding problems of vaccination. The earlier experience with changing formulas of polyvalent vaccines and their actual production had developed a mobility and capacity for adjustment in the laboratories of our commercial producers. A long lag could be expected between receipt of a native virus and its transfer into effective production of potent vaccine in sufficient amounts to close the large gap in immunity. This apparently was true of efforts in Russia with live virus vaccine as well. It was learned that the interval could be sharply reduced.

It was the end of May when the virus was distributed to manufacturers in the United States. In July, field trials with vaccines of different concentrations were begun. In August, 500,000 doses of moderate potency were distributed; in September, 14 million doses; and by the end of October, 42.6 million ml. had become available in bulk. In that month a shift to a doubled strength was possible so that, in November, 2 million ml. of 400 chicken erythrocyte agglutination units (CCA) and in December 13 million ml. were released. This was a tremendous display of emergency development power, and the lag period was sharply reduced from that of previous tests. But it was also learned that the processes are not fast enough to meet a faster moving epidemic.

Regardless of whether vaccine will be the ultimate answer to prevention of influenza, it is our major preventive reliance until something better can be produced and shown to be effective. The continued studies have abundantly shown its effectiveness. It was clearly demonstrated again in 1957, and the military experience in 1960 was an outstanding success. The effort to combine the range of antigens into a single preparation is a worthy undertaking. The value of adjuvants in promoting this objective recommends their use. As more is learned of the chemical isolation of viral antigens, the probability increases that the purified entities can be assembled into practical vaccines. It is my belief that this is more than a theoretical idea but one for concentrated prosecution. If recurrent strains are of fixed origin, the probabilities of successful development would be even greater as time revises their nature. Otherwise, I believe that we can develop strains of our own making which will encompass the antigenic range of influenza viruses. Maassab's demonstration of the production of modified viruses by active ribonucleic acid points in this direction, and so do the studies of recombination.

In any event there is little reason to hamper the development by specious arguments that it can't be done; greater effort should be made in this direction. We are the informed, the specialists, and it is our duty to impress the value of

vaccine research rather than express dilatory and specious reservations. Attention should be constantly given to those special groups with increased risks of mortality. Like the problem of Pneumococcus again, chemotherapy and chemoprophylaxis need more intensive study. Pessimism is commonly expressed as to this outlook. We should not be governed by one remark regarding the investigations of the Kefauver Committee that an ounce of prevention costs $30.00. The studies in tissue culture and some in experimental animals have been hopeful but short of practical aplication. Nevertheless, I will wager that, if greater attention is given to *in vivo* study than to *in vitro*, success in this field will come so as to overcome the immunologic complications of serologic variation. And concentrated effort should be directed to it.

The major part of what we have learned is that we must learn a lot more before we can adequately take charge.

If we take the point of view that it is the severe illness and fatality that cause the major concern, then we must emphasize the preventive aspect to those of higher susceptibility. They should avoid sick children; they should be vaccinated. If confronted with the problem of illness, *treatment* must be improved. But how? Apart from recognition of the primary cellular injury in influenza, we are quite unprepared to discuss the pathologic physiology represented by the symptomatology. The basis of the general toxicity, of the severe myalgias, is not known. But, more importantly, the mechanism of the edematous outpouring in the lungs is unexplained. My assumption has been that the epithelial injury results in toxic products which act upon capillary permeability. This, in turn, releases fluids which interfere with oxygenation, and thus more fluid is excited. In the cardiac patient, the defect is already present and the irritating phenomenon incites further difficulty, et cetera. Certain pulmonary diseases or pregnancy which may limit vital capacity present a similar initial risk. In fact, influenza virus infection in a community tends to create irritation and special risk to certain types of persons somewhat parallel to that created by severe industrial smogs. The high fatality this year in Los Angeles, and in 1936–1937, and the London and Liverpool experiences suggest a basic reaction of similar pathologic portent between viral and chemical smog.

We must be careful not to create too complete a fixation on the role of staphylococci. It may merely be "the bug of the times," and others of serious portent can be expected.

Valuable aids to treatment could be provided by materials counteracting the physiologic injury at the pulmonary and vascular level. Control of cardiopulmonary incompetence would, undoubtedly, reduce those sources of fatality. One should consider study during an epidemic of persons already known to be affected in this way. The entire physiologic disturbance of the acute infection is largely unexplored; but this is true of acute infection generally. The question remains whether there is a primary cardiac injury, or only a stress, which brings latent disturbance to attention. The vascular responses, the convalescent asthenia, and the so-called infectious shock need clarification, with hopes of therapeutic aid. Chemotherapy of this nature, or antiviral, should be sought; antitoxic principles are needed. There is the need for investigation of the role of general physiologic influences in resistance to respiratory disease. *Physiophylaxis* it might be called, but this seems far in the future. Here again, the influenza requirement is only a part of a much larger field of neglected study, but the relatively abrupt onset, the limited scope of the disease, the availability of susceptible animals of size, the capacity to grade the severity of the experimental disease or to add complicating factors strongly suggest influenza as a good working model. The experimental model could well serve for more investigation of viral-bacterial interplay—of the influence upon congenital malformations. Many other suggestions have been listed.

In any conference of this type there is always a risk of expected concurrence or pressure for concurrence as if a vote would settle the unsettled problems. And in concern for our current concepts, we may overlook promising opportunities and new approaches.

Recently I read the story of the three well-illuminated jovial souls who arrived at the railroad station just as the train was pulling out. Two of them managed to get on, while the third was left behind. One of the platform guards saw him standing disconsolately watching the train disappear. The guard said, "I'm awfully sorry you missed your train." The disconsolate one shook his head and said, "My friends will be sorry, too, for they came down to see me off."

So, if in these remarks I missed the train, I hope you'll have had a pleasant ride.

STRAIN CHARACTERISTICS

FRED M. DAVENPORT, *Chairman*

STRAIN CHARACTERISTICS OF ASIAN INFLUENZA VIRUS[1]

ROSLYN Q. ROBINSON

Identification of Variant

The earliest laboratory information obtained indicated that the agent responsible for widespread epidemics in the Far East was a type A influenza virus with significant immunologic differences when compared with earlier prototype strains (1). That the new variant was a type A influenza virus was clearly established by demonstration of significant increases in antibody titer in paired sera collected during the Asian outbreak using the complement-fixation test with an A1 antigen. In addition, it was possible to detect significant increases in complement-fixing antibody titers in paired sera collected from cases of influenza prior to the Asian outbreak, using the Asian virus as antigen. Due to the type-specific reaction in the conventional complement-fixation test, it was evident that there was no difference in the soluble antigen components of the Asian virus and earlier type A strains.

Antigenic Composition

The hemagglutination-inhibition test, which is strain specific, revealed no antigenic relationship to earlier strains using ferret or chicken antiserum prepared against several isolations made during the period 1933 to 1957. Although it is an outstanding property of influenza viruses to change in antigenic constitution over a period of years, it is perhaps evident that a more distinct change was noted in the evolution of the Asian variant than was the case in the shift from swine to type A or from type A to A1 in that antigenic relationships among strains of swine, type A, and A1 viruses are demonstrable using specific animal serum (2). There is evidence, however, that the Asian set of viruses does indeed share some specific antigenic components with influenza viruses which have been prevalent in other years. It has been demonstrated by Davenport and associates (3) that the pattern of antibody distribution determined at the present time in various age groups would provide a serologic picture of past infection with antigenic variants of influenza viruses. It is shown that in the early years of life the antibody spectrum is narrow and becomes progressively broader in later life through repeated experiences with influenza viruses. Not only is there acquisition of antibody against currently prevalent strains, but there is a reinforcement of antibody of greater magnitude against the initial infecting strain in childhood. Excellent support was obtained for establishing swine influenza virus as the possible cause of the pandemic of 1918 and 1919.

In the Asian pandemic, the first of such observations was made by Mulder and Masurel (4) who found antibody to Asian influenza virus, prior to the time the pandemic reached the Netherlands, mainly in serum collected from persons more than seventy-one years of age. This suggested that the 1957 pandemic was a repetition of the one at the end of the last century and possibly of the pandemic of 1889–1890. Nelson and Lewis (5) are of the opinion that the Asian influenza virus and the swine strain share a common antigenic component, based on the finding that infection with the Asian strain stimulated a concurrent rise in swine antibody in persons of all ages. Antibody response to swine virus in children and young adults was believed to be of a primary type, while the response in persons more than thirty-five years of age was of a secondary type. However, in some younger persons, swine antibody was detected in the absence of Asian antibody, and this was attributed to the possibility that type A and A1 strains prevalent after 1935 also share minor antigenic components with swine virus, but the relationship is not nearly as close as that of Asian to swine.

Further evidence for the sharing of minor antigenic components among various strains within type A viruses is presented by Henle and col-

[1] From the Virus and Rickettsia Section, Communicable Disease Center, United States Public Health Service, Department of Health, Education, and Welfare, Atlanta 22, Georgia.

leagues (6). By use of the strain-specific complement-fixation test, they found that, with repeated infection of guinea pigs with the same strain or with strains isolated in various years but excluding the Asian strains, the response gradually broadened to include antibody reactive with the Asian strain. Further study by this group revealed cross reactions of the Asian strain and antiserum prepared in guinea pigs against forty-one strains isolated in Philadelphia in 1951 and 1953. In addition, the WS strain isolated in 1933 reacted with the antibody prepared against the Japan/305/57 strain (7). It was concluded that antibody to Asian virus need not denote infection with that particular virus, but could be produced by multiple infection with other strains.

Sensitivity to Antibody and Nonspecific Inhibitor

Apart from the unique antigenic composition of Asian influenza viruses, probably the most outstanding variation was the range of sensitivity to specific antibody and nonspecific inhibitor. These two characteristics have been most thoroughly studied, although uniform results have not been obtained in all laboratories. Since such studies have been undertaken in many areas of the world, using different strains of virus, it would be difficult at present to draw detailed conclusions from accumulated data. From a general standpoint, however, isolates could be grouped together on the basis of sensitivity or insensitivity to specific antibody and nonspecific inhibitor (8).

That strains of Asian virus were sensitive to normal inhibitory substances in serum was immediately evidenced when it was observed that titers of acute serum specimens were extremely high, as were titers in normal ferret or chicken serum. Treatment with heat and trypsin, commonly used for removal of nonspecific inhibitor in animal serum to which some earlier type A strains were sensitive, was ineffective for use with sensitive Asian strains. Of 188 strains submitted to the International Influenza Center for the Americas in 1958, 119 were found to be sensitive to nonspecific inhibitor, and, although the major portion was sensitive to specific antibody, some variation was noted. Sixty-nine strains were found to be insensitive to inhibitor and also varied with respect to sensitivity to specific antibody. Variation in sensitivity to specific antibody in the latter group was of a greater magnitude than with inhibitor-sensitive strains. The first strains studied in our laboratory were insensitive to inhibitor but in addition were insensitive to specific antibody as well. The prototype strain A2/Japan/305/57 (egg line) was passaged in ferrets and mice with subsequent passage in eggs, with the result that a line was produced which was quite sensitive to nonspecific inhibitor (9). In order that the strain could be used in the hemagglutination-inhibition test, a variety of procedures was used in an effort to destroy nonspecific inhibitory substances and reveal true antibody titers. This was accomplished by potassium periodate treatment of serum (10, 11) and was recommended to the network of laboratories collaborating in the World Health Organization influenza program. Although at a later date a small number of strains were encountered which were sensitive to specific antibody and insensitive to nonspecific inhibitor, the recommendation was not changed in view of the fact that we had not observed alteration in specific antibody titers as a consequence of resorting to the use of potassium periodate.

Further, after instituting the use of potassium periodate in the network of collaborating laboratories, it did not appear warranted to risk further possible confusion at that time. It is probably ideal, however, to make use of an antigen which would require no treatment of serum other than heat in order that the objection to chemical treatment of serum might be eliminated. However, considerable variation has been found among inhibitor-insensitive strains with respect to their sensitivity to specific antibody and, in addition, there is disagreement concerning the stability of inhibitor-insensitive strains to detect specific antibody in all serum. In an extensive study, Fukumi (12) observed wide variation among 300 strains of Asian viruses in their sensitivity to antibody in human serum. Similar results were obtained when specific rooster antiserum was used and, in addition, considerable correlation was found between reactivity to antibody in human serum and reactivity to antibody in rooster antiserum. Those strains showing higher inhibition titers in one case also reacted in high titer in the other. Reactivity with nonspecific inhibitor was also observed but no correlation of this property could be demonstrated with reactivity in human or rooster antiserum. Further studies indicated that

the degree of reactivity with antibody in human convalescent serum as well as post-vaccination serum was constant and was not dependent upon a single human serum. Strains isolated from cases in a circumscribed outbreak did not differ significantly except in sensitivity to nonspecific inhibitor. Cross-absorption experiments with specific rooster antiserum were performed with three strains exhibiting wide variation in sensitivity to antibody. No positive evidence was obtained to indicate that any of the strains exhibited specific differences in antigenic composition.

In similar experiments, Cleeland and McKee (13) determined the reactivity of twelve inhibitor-insensitive strains, including the A2/Japan/305/57 egg line, for antibody in twenty paired serum specimens from humans convalescing from Asian influenza. As in the work of Fukumi (12), the strains collected in one geographic area over a short interval differed markedly in their capacity to detect antibody, ranging from six of the twenty to nineteen of the twenty paired serum specimens. In contrast to the studies of Fukumi, the variation among strains was attributed to antigenic variation in view of the fact that no distinct pattern of response could be obtained. All serum showed a rise in antibody titer to at least one strain; serum from a single patient reacted differently to various strains; and in a single strain high titers were detected in some serum but low titers, or no titer at all, in others. Further studies are necessary to determine whether variations in results are due to P-Q variation, distinct antigenic variation among viruses of the same subtype, or to other factors not as yet considered.

Other Characteristics

Many investigators have contributed information concerning properties of the Asian influenza strains as compared with strains isolated in previous years. The A2/Japan/305/57 strain was found to consist primarily of virus in filamentous form (14), which is characteristic of newly isolated type A strains (15). As with the earlier type of A viruses, the Asian strains were found to propagate readily from throat washings when inoculated into embryonate eggs via the amniotic route. Following isolation, the viruses grew in the allantoic cavity, yielding virus with hemagglutinating titers of 1:40 to 1:320, using chicken or guinea pig erythrocytes and at room temperature or 4°C. (1, 2). The magnitude of the hemagglutinating titers differed somewhat from earlier type A isolates. Although not without exception, Asian strains were found to agglutinate erythrocytes to a considerably lower titer than generally possible with earlier type A strains. It was also found that the A2/Japan/305/57 strain agglutinated sheep cells in the complement-fixation test. The agglutination of sheep cells is a recognized property of the B/Lee/40 strain. The Asian viruses were subsequently shown to agglutinate a wide spectrum of erythrocytes. Wang and Lin found that erythrocytes from seventeen animal species were agglutinated by the new viruses, whereas only eight to nine species were agglutinated by earlier type A strains. While the B/Lee/40 strain agglutinated eleven of the seventeen species of erythrocytes, it was the opinion of Wang and Lin that the very broad spectrum of agglutination was a unique characteristic of Asian viruses (16).

Isolation of Asian influenza viruses in monkey kidney cultures has been found to be superior to isolation in embryonate eggs in some laboratories (17) and inferior in others (11, 18). It is possible that the ease with which isolations were accomplished in various laboratories was dependent upon differing experimental conditions. However, certain characteristics of Asian strains have been noted after isolation in tissue culture which were not found consistently after isolation in embryonate eggs. Mogabgab and associates (18) found that differences between tissue culture and egg line viruses were manifest only by the decreased affinity of tissue culture lines for sheep erythrocytes. However, tissue culture lines insensitive to specific antibody were not encountered. All strains were sensitive to nonspecific inhibitor and, in this respect, resembled the A2/Japan/305/57 EFME line with the exception that receptor-destroying enzyme was sufficient for destruction of nonspecific inhibitory materials. In addition, it was possible, with tissue culture line antigen, to demonstrate significant increases between acute and convalescent antibody titers with the hemagglutination-inhibition test in all cases in which rises were demonstrable by the complement-fixation test.

Soon after isolation, the A2/Japan/305/57 strain was found not to be particularly virulent for mice in that only minimal pulmonary lesions

were obtained on primary or secondary passage in mice inoculated intranasally (1). Jensen attributed characteristic pulmonary consolidation obtained in early mouse passage to toxicity, since, after the fifth passage, lesions were no longer found (2). Extended studies on mouse adaptation were done by Wen and Wang, who found that symptoms of illness and areas of pulmonary consolidation were seen in the first passage but that, thereafter, through the sixth passage, only focal microscopic lesions were observed. Signs of adaptation were first observed on the seventh or tenth passage and further passage resulted in increased symptoms, pulmonary lesions, and death (19). It would appear that the behavior of Asian strains of virus is similar to that observed for other newly isolated type A strains.

Summary

It is evident from data available at present that the Asian variant resembles earlier type A strains in many respects, but differs significantly in some characteristics. Outstanding among the differences is a distinct antigenic composition accompanied by a wide variation in sensitivity to specific antibody and nonspecific inhibitor. In addition, the spectrum of species of erythrocytes which are agglutinated by Asian strains appears to be broader than for other type A viruses, and the hemagglutinating titers are generally lower. While it is not possible to draw a comparison between Asian strains and earlier type A strains with respect to isolation and growth in tissue culture, it is apparent that isolation in monkey kidney cells does not yield a strain of virus identical in behavior to that isolated in the chick embryo.

While much information is already available, much additional study is necessary before a complete understanding of the behavior of the new variant, as well as possible relationships to earlier type A strains, may be realized.

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General Discussion

Dr. Hideo Fukumi: We examined about 300 strains of A2 influenza virus and found wide variations in hemagglutination-inhibition against human convalescent serum; namely, some of them showed high hemagglutination-inhibition titer, whereas others were not inhibited at all. But, in general, the titers shown by the strains against a human convalescent serum were shown to be distributed around a maximal peak by a skewed distribution curve. The same strains behaved similarly but on a somewhat smaller scale, when tested against rooster antiserum obtained by immunization with one of three strains (Adachi, K9, and Y5) which were selected as representative, respectively, of high or low antibody avidity. These two series of variations were fairly closely correlated.

We further selected eight strains as representative of the range of inhibition variation and tested hemagglutination-inhibition titer of a number of human convalescent sera against them. The degree of the inhibition variation of these strains was considered to be a general property and not influenced by the individual nature of any one serum. This held true also when sera from persons inoculated with monovalent Adachi vaccine were employed.

That the variation here mentioned was based upon the difference in avidity of the strains to the antibody was evidenced by antibody absorption tests. Antibodies either in the human convalescent serum or in the specific rooster serum were most readily absorbed by well-inhibited strains but less easily by the strains not easily inhibited.

It was found that the immunizing potency of vaccine prepared from strains of higher antibody avidity was better than that of lower avidity according to animal experiments. Mouse adaptation might have an influence upon the immunizing potency of some strains, especially for those of lower antibody avidity, so as to increase the potency to the level of those of higher avidity.

The details of these studies have been published in the *Bulletin of the World Health Organization* (1959, *20*, 421).

Dr. M. R. Hilleman (West Point, Pennsylvania): I wish to confine my remarks to a brief discussion of three aspects of the Asian influenza strain problem referred to in Dr. Robinson's excellent review: (*1*) certain important factors essential to the early detection and identification of a new Asian pandemic influenza virus; (*2*) some practical considerations in the application of the diagnostic hemagglutination-inhibition test; and (*3*) comments relating to the interpretation of data which suggest an antigenic relationship between the Asian influenza virus and the agent which caused the 1889–1890 pandemic.

The researches of nearly three decades on influenza, culminating with the pandemic of 1957–1958, have clearly shown that, even if there is a finite limitation to the extent of possible antigenic variation of influenza virus and even if new antigens cannot be created by mutation *de novo*, we have not as yet identified all of the possible antigens of influenza virus and put them into a single vaccine which is all-protective. It seems unavoidable, therefore, that for the indefinite future and, indeed, for the next pandemic, we are going to have to continue to depend upon the early detection and rapid identification of the new and different virus well in advance of its pandemic spread if we are going to be able to prepare vaccines early enough to do any appreciable amount of good.

In the detection of new influenza virus strains, one needs first to know that the disease exists and, to accomplish this at the earliest time, one must not confine himself to scientific sources of information alone. One lesson of the last pandemic was that the first tip-off of the occurrence of Asian influenza did not originate from a scientific agency, at least insofar as the Western Hemisphere was concerned. Instead, our first indication of the event was a brief report in *The New York Times* of April 17, 1957, which described an outbreak of influenza in Hong Kong in which there were an estimated 250,000 cases among the 2,500,000 population. This high attack rate, 10 per cent, alerted us at Walter Reed Institute of Research to believe that something unusual was afoot and, accordingly, we asked the 406th Medical General Laboratory in Zama, Japan, to investigate and recover the virus if possible. On May 13, just twenty-five days after the report in *The Times*, the first Asian influenza virus was received in Washington, D. C., for analysis.

Once a newly recovered virus is at hand, the laboratory must have the capability for rapid identification so that the virus can be placed either in the category of the new and unique or relegated to the category of the past. The laboratory must also know what kind of data and how much are needed to formulate a definitive and correct conclusion, lest it unintentionally touch off a false alarm.

In our laboratory,[1] the most rapid and simple method for establishing the Asian virus as type A was in tests for group-specific soluble complement-fixation antigen in Asian-influenza-infected egg

[1] Meyer, H. M., Jr., *et al.*: Proc Soc Exp Biol & Med, 1957, *95*, 609.

TABLE 1

HEMAGGLUTINATION-INHIBITION AND COMPLEMENT-FIXATION TESTS OF PAIRED SERA FROM CASES OF INFLUENZA IN FAR EAST AND IN RECENT A1 OUTBREAKS IN TESTS WITH ASIAN AND PROTOTYPE A1 INFLUENZA VIRUS

Influenza Outbreak	Patient	Serum Sample	Hemagglutination-Inhibition		Complement-Fixation	
			A/Japan/305/57	A Japan/301/56	A/Japan/305/57	A/Hawaii/303/56
Far East, 1957	1	Acute	<5	10	5	5
		Convalescent	40	10	40	80
A1, 1956	2	Acute	5	10	<5	<5
		Convalescent	10	160	320	320

TABLE 2

COMPARISON OF ASIAN INFLUENZA STRAIN A/JAPAN/305/57 WITH PROTOTYPE STRAINS IN HEMAGGLUTINATION-INHIBITION TESTS WITH CHICKEN ANTISERA

Chicken Antiserum against:	Hemagglutination-Inhibition Titer against:	
	Homologous Virus	A/Japan/305/57
A/Japan/305/57	(Titer <1:25 in tests with prototype strains)	400
A/Japan/301/56	400	0*
A/FLW/1/52	800	0
A/FW/1/50	400	0
A/FM1/47	800	0
A/PR8/34	1,600	0
A/WS/33	400	0
A-Swine	3,200	0
B/Va/301/55	400	0
B/Lee/40	1,600	0
C/1233/47	400	0
D-Sendai	1,600	0

* 0 titer equals less than 1:25.

membrane extracts using paired sera from past cases of influenza A and B (table 1). Likewise, the best method for rapid strain analysis was by the time-proved hemagglutination-inhibition technique using sera from chickens immunized with prototype strains of virus which had been passed only in embryonated eggs and which represented the past antigenic spectrum of influenza (table 2). Such tests were performed in a matter of hours and the findings were confirmed later in tests with convalescent sera from ferrets infected with these prototype strains (table 3). There was no need for "fancy work" and no need for serum absorptions. By this test procedure, a sixteen-fold or less titer of prototype serum tested with the new virus as compared with the homologous titer appears of considerable significance in relation to immunization of man by means of vaccines. The etiologic relationship of the newly recovered Asian virus to the disease in the epidemic was established by means of hemagglutination-inhibition tests of paired sera from cases in the pandemic (table 4).

Once the striking antigenic difference from previous prototype influenza virus strains was established, it was important to inquire into the antibody status of adults in the contemporary time period. Such study showed an alarming deficiency of protective antibody against the Asian virus compared with the previously occurring strains and suggested that, indeed, the human population was inexperienced with the new agent (table 5).

Finally, then, the reported high attack rates for the disease, the unusual seasonal occurrence of the epidemic in the early warm period of the year, and the laboratory findings convinced us that a potential influenza pandemic was in the making and we felt justified on May 22, 1957, just thirty-six days after appearance of *The New York Times* article, to notify the World Health Organization, the United States Public Health Service, the Armed Forces Epidemiological Board, and the Armed Forces Medical Departments of the findings so

TABLE 3

COMPARISON OF ASIAN INFLUENZA STRAIN A/JAPAN/305/57 WITH PROTOTYPE STRAINS IN TESTS WITH FERRET ANTISERA

Ferret Antiserum against:	Hemagglutination-Inhibition Titer against:	
	Homologous Virus	A/Japan/305/57
A/Denver/2/57	640	0*
A/AA/1/57	640	0
A/AA/4/56	320	0
A/NED/36/56	320	0
A/Sendai/5/56	160	0

* 0 titer equals less than 1:20.

that mobilization of defenses could be initiated. In all of this, the need for early detection, for available reagents for laboratory studies, for simple methodology, and for speed was clearly emphasized.

In his discussion of laboratory diagnosis of influenza by the hemagglutination-inhibition method, Dr. Robinson has suggested that it may be ideal to employ antigens requiring no treatment other than heat to remove nonspecific inhibitors. I am in total agreement with the concept and I want to emphasize the importance of this simplification of technique. Among the first seven Asian influenza viruses studied at our laboratory[1] at Walter Reed Army Institute of Research, two of these proved essentially unaffected by nonspecific inhibitor in human sera. The A2/Japan/305/57 virus retained this desirable characteristic in successive passage in embryonated eggs, and such virus was sufficiently antibody-avid to provide a positive diagnosis in all cases in which influenza was suspect based on other determinations. In certain other laboratories, the virus was passed in animals in order to increase the avidity of the virus for reaction with antibody. At the same time, however, this rendered the virus exquisitely sensitive to nonspecific inhibitor. Hence, the time-consuming step of treating serum with sialidase (RDE) or with periodate to remove inhibitor had to be employed. Additionally, a harmful effect[2] was introduced in the excessive destruction of antibody by these reagents in serum with low antibody titers (*see* table 4). The result of this was the addition of an unnecessary step which added a hazard to the diagnostic effectiveness of the test.

The question remains unanswered at this late date whether the principal antigen (or antigens) of the Asian virus was a new component(s) arising through genetic mutation or whether it represented the re-emergence into prominence of a minor antigenic component present in earlier influenza viruses. Mulder,[3] in his demonstration of antibody against Asian virus in sera from persons eighty to ninety years of age, has suggested that the pandemic of 1957 may be related etiologically to the pandemic of 1889–1890, which also had an Asian origin. Tests in our laboratory[2] of untreated sera from persons of different ages have shown a gradual increase with age in the mean hemagglutination inhibition titer and in percentages of positive sera for Asian influenza virus up to the group fifty to fifty-nine years of age, with little change in the older groups (figure 1). These data favor the cautious interpretation that the increased amount of antibody in older persons may be due more to experience with a wide composite of influenza virus antigens present *also* in Asian virus than with the Asian virus itself. Experimental support for this concept has been shown by Henle and associates[4] who, by use of the strain-specific complement-fixation test, found that guinea pigs infected repeatedly with the same strain or with strains of progressively later vintage gradually developed a broadening antibody response which embraced strains not included in the infecting strains, e.g., the Asian strain.

In summary, the experience of the Asian pandemic has clearly shown how the causative virus can be detected early, how effective diagnostic

TABLE 4

EFFECT OF CHOLERA FILTRATE AND PERIODATE ON NONSPECIFIC INHIBITOR AND ON INFLUENZA ANTIBODY IN PAIRED HUMAN SERA

Case	Kind of Infection	Specimen	Homologous Hemagglutination-Inhibition Titer		
			Untreated Sera	Cholera-Filtrate Treated	Periodate Treated
1	Far East	Acute	<5	<5	<5
		Convalescent	40	<5	<5
2	Far East	Acute	<5	<5	<5
		Convalescent	40	10	10
3	A1	Acute	40	10	20
		Convalescent	320	320	320

TABLE 5

ANTIBODY STATUS OF THIRTY AMERICAN MILITARY CADRE PERSONNEL FOR ASIAN INFLUENZA VIRUSES AND FOR PROTOTYPE A1 AND B VIRUSES

Serum Titer	Number of Sera* with Titer			
	Far East—A/Japan		Prototype	
	305	307	A/Japan/301/56	B/IB1/50
0	30	27	6	0
1:5	0	2	6	1
1:10	0	1	8	4
1:20	0	0	6	12
1:40	0	0	3	9
1:80 or >	0	0	1	4

* Sera were treated with cholera filtrate to remove non-specific inhibitor.

[1] Mulder, J.: Lancet, 1957, *2*, 334.

[2] HILLEMAN, M. R., *et al.*: New Engl J Med, 1958, *258*, 969.

[4] HENLE, W., *et al.*: Lancet, 1958, *1*, 818.

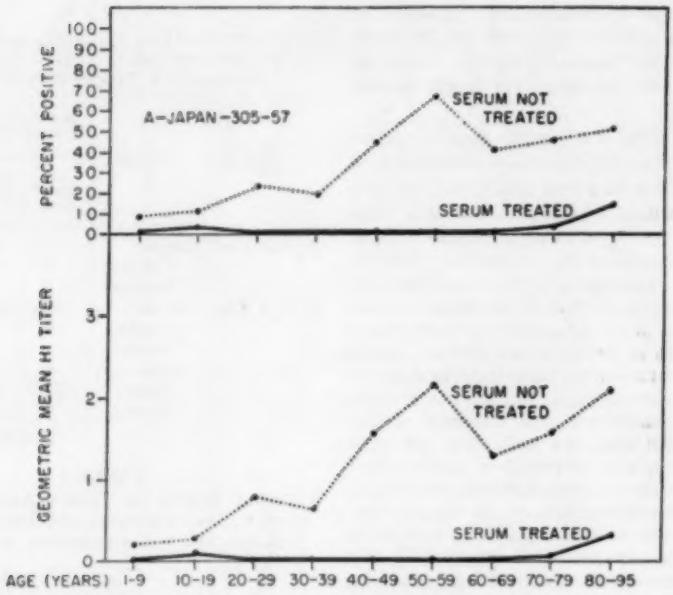

FIG. 1. Age-specific distribution of the hemagglutination-inhibiting (H.I.) substances against Asian influenza virus in sera treated and not treated with cholera filtrate.

tests can be developed readily, and how the disease can be effectively prevented by use of a vaccine prepared from the pandemic strain. In the absence of indication that influenza virus will, in the future, lose any of its capricious quality for antigenic change, it remains essential and expedient for continued influenza surveillance by the worldwide network of laboratories of the World Health Organization, by the Armed Forces, and by other groups. It is only by continued effective operation of such activity that the next pandemic virus can again be apprehended in advance of its pandemic spread.

DR. C. H. ANDREWES (Mill Hill, England): I want to discuss only one matter and that is the implications of the strain characteristics of Asian influenza with regard to possible origin of the virus.

Dr. Robinson and others have called attention to various differences between Asian influenza and earlier strains. Quite a lot of workers have been publishing notes about all sorts of differences. Obviously, the most important characteristic is the presence of this peculiar A2 antigen as the dominant antigen of the new virus.

The crossing which is sometimes seen and which has been reported with earlier strains may be due to the fact that these strains have a little bit of this A2 antigen or it may simply be that they have some other minor antigen in common. There are various nonspecific inhibitors which react differently with the Asian viruses and the other viruses. Then there is the fact that the Asian viruses agglutinate a much wider range of animal erythrocytes than do the earlier strains. The P-Q variation has been much more prominent and troublesome with the Asian strain than with earlier ones. The O-D variation is very much less prominent with the Asian strains than with the earlier ones. And, finally, the Asian viruses seem to have a greater antigenic stability than the A1 strain. Maybe this is very important. My colleague, Dr. Isaacs, tells me he can't detect any very striking differences between the Asian viruses isolated in 1957, 1958, 1959, and 1960.

If it turns out that this virus is very much more stable than the others, we must recall that influenza apparently keeps itself going by virtue of its ability to vary, and we might take hope that in the decade to come we are going to have less bother from influenza once it has gone right through the population and affected all susceptible members. I do not hold out too strong hopes about that. What I do want to emphasize is that we have here a whole range of characteristics in which the Asian virus differs from the other ones. Now it is just conceivable that some mutation might so affect the constitution of the virus that it would be differ-

ent in all of these respects, but I find it a little difficult to believe. It seems to me to indicate rather that the virus has originated from somewhere other than in the pre-existing A1 viruses. Now, if you ask me where it came from, I don't know. I must admit that I am very attracted by the hypothesis that Professor Mulder put forward, that it may have come from some animal reservoir.

Attempts to get concrete evidence in favor of any particular animal reservoir have been completely negative. But, so far, attention has been devoted entirely to investigating pigs and horses and, on mature reflection, I think it more likely that, if there is an animal reservoir, it is in some very much more obscure animal than these. Eastern equine encephalomyelitis "spills over" and affects horses and man when there has been a build-up of activity in its natural hosts. And it seems to me possible that this sort of thing is going on somewhere in this mysterious Central Asia. When this happens at intervals of a few decades, it may spill over to man. The difference between influenza and the equine encephalomyelitis is that the former has the ability to go on passing from man to man.

It has been very difficult to get any information out of China about what actually happened in Kweichow Province. I have been in communication with Dr. Chu and he doesn't seem to be able to get any information. I should like to suggest to Professor Soloviev that, if this thing has come out of Central Asia, it may come out of the Soviet Union just as easily as out of China, and it is up to him to organize some expedition to look for obscure animals and see whether he can't find an influenza virus in them.

Dr. Steele—or was it Dr. Kaplan—yesterday was very impressed with the glamor of looking for something in some species other than man. It seems to me you could hardly conceive anything more glamorous than an expedition into Central Asia to look for epidemics of influenza in marmots or prairie dogs or something of that sort. It's a long shot, but if it came out it would give you a clue how to stop future pandemics of influenza which would be enormously more effective, enormously cheaper than the enormous amounts of money which are spent in the present orthodox approach to the subject.

Dr. Fred M. Davenport: Yesterday I had the amusing experience of watching Dr. Francis being very uncomfortable as one speaker after another referred to a point he had hoped to use in his talk last evening. As he jokingly said, "There goes ten more pages." Today, the joke's on me in that, while sitting here as chairman, there have been many things stated that would make an obvious introduction to the paper I am supposed to give this afternoon. However, we will not deviate from the scheduled program.

I would like to make one or two comments before opening up the discussion from the floor. First, in the two large studies on serum antibody—that of Dr. Mulder and our own—Asian antibody was present in sera before the epidemic struck when over a thousand serum specimens were examined. In our own case the sera were drawn so that an equal number of specimens was obtained from each age group. In this collection, it was obvious to us that the pre-epidemic presence of Asian antibody was not an effect of broadening, but there was a peculiar high frequency of high levels in the sera of persons of more than seventy. Moreover, as Dr. Francis mentioned last night, the serum antibody absorption characteristics of these individuals identify the Asian antibody as a dominant one in their specimens.

Second, I too am in favor of simplification of the hemagglutination-inhibition test when it can be done. However, it is clear that heat alone is not the answer despite its apparent attraction because, as has been shown, heat changes the capacity of antisera to react with some of the Asian antigen, a characteristic we had not noticed before, and this property can be restored by the addition of appropriate solutions of guinea pig serum. We are obviously not adding back antibody.

We were unable to confirm the observations by Nelson and his colleagues in Australia that the sera of children show swine antibody after infection with Asian strains. In our experience, this was characteristic only of the older persons.

The time is short and we have ten minutes for a general discussion. I would like to ask Dr. Magill, whose interest in the problems of antigenic variation is a long one, if he would care to comment.

Dr. Thomas P. Magill (Brooklyn, New York): Really, I do not care to make any comments about the problem of strains. However, there are two other points on which I would like to comment.

I want to congratulate Dr. Loosli and the Public Health Service for having conjured up, out of the blue, a new disease.

Also, I have been intrigued by Dr. Andrewes' suggestion that the possible relationship of the A2 strains to animal strains might be indicative of the origin of the outbreak of influenza, particularly in view of the suggestive indictment of the animals of China. It seems that always some far away place is indicted. Epidemics seem never to arise within our own borders.

Dr. A. Frederick Rasmussen (Los Angeles, California): In the last few years we have stumbled on an interesting relationship between the sialidase

(RDE) of animal A2 strains. As you know, the classical A strains, if exposed to physical agents like heat or ultraviolet light, show a characteristic pattern of destruction of various components of the viral particle in which first the infectivity, then the RDE activity, and, finally, the capacity to adsorb to and hemagglutinate cells is lost in that order.

The A2 strains differ in that, if these viruses are heated, the hemagglutinin is destroyed before the RDE. In a preliminary study we had occasion to study swine, and swine alone of earlier strains also have this quality. When we became aware of the interest of Dr. Andrewes and others in the possible animal sources, we extended this study to include other animal strains. We have now had an opportunity to study the Czech duck strain, the equine strain, and the British duck strain. It is not possible to study fowl plague in this country. But, fortunately, a few years ago, Dr. Brandly obtained a strain of virus which was supposed to be related to NDV. Actually this virus shares the soluble antigen of the A viruses and, as Hallauer and others have shown, it also shares surface antigens with fowl plague. It is certainly not classified as typical fowl plague but it belongs to the fowl plague complex. This virus, also, along with the equine and the duck strains, has this unique characteristic in that the hemagglutinin is less stable to heat than the RDE, and this again offers some evidence that the A2 strains have some fundamental biologic relationship with the animal strains.

Dr. Morton Klein (Philadelphia, Pennsylvania): I would like to make a comment indicating that the detection of antigenic relationships between Asian influenza virus and prior strains of influena virus might be dependent on the origin of the Asian strain used. We collected sera from a group of subjects (ages twenty to twenty-five) prior to the Asian epidemic and determined their neutralizing activity in chick embryos and hemagglutinating activity against a nineteenth egg passage chick embryo strain of the Japan 305 strain of Asian influenza. No antibodies were detectable in this age group, as has been noted by others. However, when we used as antigen throat washings obtained directly from patients, we found that approximately 50 per cent of the sera had neutralizing antibodies using the amniotic sac of the chick embryo as the test site. The results indicated that for the detection of minor antigenic components the definitive reference antigen should be the virus as it grows in the throat, for, following chick embryo passage, certain minor antigenic components may be lost.

Dr. Davenport: May I have a volunteer to answer this question? It would seem to me that, first, the person must be asked whether or not he believes this is antibody that he measured, or is it a nonspecific inhibitor? I am sure those of you who have studied the problem realize that nonspecific inhibitor will also nonspecifically neutralize the Asian strains. Also, since I have the microphone and in rebuttal to Dr. Andrewes, I would like to make a statement in defense of the theory that the A2 is derived from A1 and the A1 from A. As the A2 became prevalent, the A1 seemed to disappear. In fact, as far as I know, it has. The A disappeared from the world when the A1 made its appearance. This suggests to me that, rather than there being an animal reservoir, each of these was derived from its predecessor. It further suggests that at some time the transfer of influenza from the human throat must be limited to relatively few throats and that we do not have influenza going all year round in a large number of people.

Dr. Berlin is not in the audience, is he? If I had brought with me six slides, illustrating some material that he has just started to bring together for publication, we could demonstrate that the statement that the inhibitor characteristics of the Asian strains are unique and have not been seen before is a bit of an over-simplification. We have found other strains from the older isolates that have some of the same peculiar properties, which leads us to the conclusion that antigenicity and inhibitor sensitivity are not correlated and can be disassociated. Therefore, it is really unnecessary to assume because of the inhibitor reactions that the current Asian strains could not possibly have had ancestors in the preceding isolates. However, as I say, I do not have the slides and that will have to remain as an undocumented statement which we hope will reach the literature shortly.

Dr. Florence Lief (Philadelphia, Pennsylvania): Hearing the previous speakers' comments on the hemagglutination-inhibition test strengthens my feeling in favor of the strain-specific complement-fixation test, which I believe our laboratory has used to advantage. We are not bothered by nonspecific inhibitors of hemagglutination, and sera do not have to be processed before use. We are not bothered by phase variations of strains or sensitivity of viruses to inhibitors. We believe that, to all intents and purposes, the strain-specific complement-fixation test matches very closely in specificity the hemagglutination-inhibition test.

However, on examining large numbers of human serum specimens, some of our complement-fixation data differ from the hemagglutination-inhibition data reported by Dr. Davenport and his group and by Dr. Mulder and others. The results presented by Dr. Robinson and Dr. Hilleman on the guinea pig work which we have done suggests that all of

the antigens of influenza virus type A (this is all we have worked with so far) are present in any given mutant. This hypothesis, I feel, was reinforced by our results with human sera, which I would like to present to you.

We had a collection of sera from an outbreak of A1 influenza which occurred before dissemination of A2 virus in this country. We isolated A1 virus from that outbreak and showed specific rises to it in a group of patients. Later we were able to go back to that group of sera and examine it for the presence of A2 antibody. We found that in an age range of from nine to sixty-five years, 38 per cent of the subjects showed A2 antibody in their convalescent sera. Now, this might be an unusual population, since it was in an institution for retarded children and, apparently, when there is an outbreak of influenza, they get it. Thus, we believe they are representative of a group of humans who have more than the usual frequency of influenza.

We have also examined prevaccination sera of another population and found the incidence of A2 antibody to range from 9 to 22 per cent, the incidence increasing with age. The age range in this group was from eleven to sixty-one years.

We also studied acute-phase sera from some two hundred cases of A2 influenza and found that again there was a gradual increase in incidence of preinfection A2 antibody with age. In groups past the age of twenty-one, it ranged between 26 and 45 per cent, being highest in the age group of fifty to sixty years. However, because the levels were very low, if we computed the geometric mean serum titers, we were able to demonstrate A2 antibody only in the fifty- to sixty-year age group. Thus, geometric mean serum titers might be misleading in representing the antibody status of age groups.

These results suggest to us that repeated exposure to type A strains might more likely be responsible for the presence of A2 antibody in the populations cited than prevalence of the Asian strain some sixty years ago. The same mechanism which finally, after the fourth inoculation of an A1 virus, caused our guinea pigs to develop antibodies to all type A strains, embracing swine, A, A1, and A2 subtypes, might well have functioned to produce the A2 antibodies in the human subjects studied.

DR. DAVENPORT: We must move on. There is another discussion period following other papers that will be concerned with some aspects of the same questions. If there is one conclusion that this conference can reach—although I'm not sure that it is supposed to reach any—it is that there are still valid differences of opinion that will make for very interesting work and very interesting discussion in the future.

METHODS OF DIAGNOSIS

FRED M. DAVENPORT, *Chairman*

LABORATORY DIAGNOSIS OF INFLUENZA BY VIRUS ISOLATION[1]

EDWIN H. LENNETTE

Definitive evidence for the viral etiology of influenza was first adduced in 1933, when Smith, Andrewes, and Laidlaw (1) succeeded in transmitting the infection to ferrets by intranasal instillation of filtered throat washings from patients with clinical influenza. Shortly thereafter, the albino Swiss mouse was shown to be susceptible to infection by nasal instillation of virus from ferret or human sources. However, repeated passage of turbinate material in the ferret, or of lung tissue in the mouse, is generally required to adapt the virus to the point where it will consistently produce marked, frequently fatal, pulmonary disease. The chick embryo, first introduced into virologic methodology by Goodpasture and his associates (2), was shown by Wilson Smith (3) in 1935 to be highly susceptible to infection with the virus. The tedium and labor connected with repeated passages in animal host systems, together with the attendant disadvantages of animal care in contrast to the ease of manipulation and care of the embryonated egg, quickly led to the almost complete replacement of the mammalian host by the embryonic avian host for experimental and applied work on influenza and its causal virus. With the recent advent of simplified tissue culture techniques, attention has naturally turned to the possible usefulness of cell cultures for the isolation and identification of the virus.

The full potentialities of the fluorescent antibody technique for the detection of virus in nasal or pharyngeal secretions have yet to be developed and assessed. Consequently, the most rapid, although not necessarily the simplest and least expensive, procedure for the specific diagnosis of influenza at the present time is the isolation of the virus from the patient. In this connection, since the chick embryo is so widely employed in diagnostic work, it appears desirable to discuss the usefulness of this highly susceptible host, and to compare it with an *in vitro* host system, namely tissue or cell cultures.

The proportion of instances in which virus is recovered from the patient varies with different outbreaks and with technical competence, and is affected by a number of factors, such as the conditions under which the specimen was obtained, shipped, stored, et cetera. Ideally, throat washings should be obtained as promptly after the onset of symptoms as possible; for practical purposes, the upper limit might be set at three days post onset. It is desirable, although not necessary, to have the patient first rinse his mouth with broth, distilled water, skim milk, or a similar medium; these mouth washings should be discarded. The patient should next gargle with 15 to 20 ml. of the diluent and the garglings should be collected in a clean, stoppered container for transport to the laboratory. The specimens should be kept refrigerated during transportation, either with wet or dry ice; in the laboratory they should be stored on dry ice until processed for inoculation into the selected host system. Penicillin and streptomycin should be added to the washings prior to use as an inoculum.

Embryonated hens' eggs of ten to twelve days' incubation are used. Inoculation is generally by the amniotic route, since the amniotic membrane is markedly more susceptible to infection with "wild" strains of type A influenza virus than is the allantoic membrane. The newly isolated strain may, after one or more additional amniotic passages, be maintained by allantoic passage, a simpler technique. Type C strains, by contrast, apparently can be maintained in the laboratory only by amniotic passage; strains of type B virus, which appear to be equally infective for both amniotic and allantoic tissues, can be recovered with equal facility by either route of an inoculation. Since in many cases, especially during the early stages of an outbreak, the immunologic type of the virus is unknown, the general practice has been to inoculate throat garglings into the amniotic cavity, usually in amounts of 0.1 ml. Our practice has been to inoculate 0.1 to 0.2 ml. by this route, although we have recently changed our standard operating procedure, which now requires that 0.2 ml. be inoculated into the amniotic cavity and 0.2 ml. into the allantoic cavity (cf. Lief and associates (4)). The fluids are harvested after forty-eight to seventy-two hours of incubation and tested for the presence of virus, as described further below. Monolayer cell cultures of monkey kidney tissue are inoculated with 0.1 ml. amounts and examined

[1] From the Viral and Rickettsial Disease Laboratory, California State Department of Public Health, 2151 Berkeley Way, Berkeley 4, California.

over the succeeding several days for the presence of virus (*see* below).

Generally speaking, strains of influenza virus are not highly lethal for the chick embryo on original isolation, the lethal propensity usually appearing only after repeated passage. In regard to monkey kidney cell cultures, only occasional strains possess some degree of cytopathogenicity. Influenza viruses may thus propagate in both host systems without readily apparent evidence of multiplication, such as death of the embryo or cytopathic degeneration of the cells in culture. Other means of detecting the presence of the virus are therefore required, and the most common approach is based on the well-known capacity of influenza viruses to cause agglutination of erythrocytes of certain mammalian and avian species. Egg fluids are tested for the presence of hemagglutinins using serologic tubes (selected for evenly rounded bottoms), or clear Lucite plates with evenly rounded depressions, or wells, to hold the reagent mixtures. Usually only qualitative screen tests are done although, under certain circumstances, time is saved if quantitation is carried out without a prior screening procedure. Tests for the presence of hemagglutinins in monkey kidney cell cultures are done in a similar manner, using the supernatant culture fluids, or may be done by the adsorption-hemagglutination technique devised by Vogel and Shelokov (5).

Amniotic fluid frequently gives false-positive agglutination patterns when used in low dilutions, and hence should be diluted at least 1:10 in saline in order to reduce the incidence of such reactions, as well as to reduce the viscosity of the original fluid. Allantoic fluid likewise should be diluted at least 1:5.

For qualitative determinations on the presence of hemagglutinins, the properly diluted fluids are delivered in equivalent volumes to three series of tubes using two to four tubes in each series. Saline suspensions of washed chicken erythrocytes are added to one series of tubes, washed guinea pig erythrocytes or human group O erythrocytes to the second series and, as a control, only saline to the third. One set of tubes of each of the three series is incubated at room temperature, i.e., approximately 22°C., the other at 4°C. for forty-five to sixty minutes. This interval affords opportunity for sedimentation of the erythrocytes.

The erythrocytes in the saline control tubes, and in those tubes containing test fluids essentially devoid of virus, have a sedimentation pattern characterized by a small, sharply outlined button. Wherever the test material, either egg or culture fluid, contains sufficient virus to produce demonstrable agglutination, the cells settle out in a pattern characterized by a disc with saw-tooth edges and readily apparent clumps of cells adherent to the sloping sides of the bottom of the tube.

While virus isolation rates as high as 90 per cent have been reported (6) through the use of embryonated eggs inoculated by the amniotic route, virus recovery rates as low as 19 per cent have been reported by others (7) using this same technique. Even when the inoculum contains virus, amniotic fluids from the inoculated eggs often do not contain sufficient virus to induce readily apparent aggregations of erythrocytes. Irrespective of the factors responsible for the wide variations in viral recovery rates, i.e., whether it be technical skill in the virus isolation procedures or inadequacy of the specimens, either through collection or shipment or storage, it thus becomes necessary to make additional passages in eggs; a minimum of three passages is generally considered necessary before the specimen can be called negative. A comparable situation exists with the use of monolayer cell cultures of monkey kidney tissue for the isolation and propagation of influenza virus. Vogel and Shelokov (5) observed that the virus will frequently grow in this cell system. However, the influenza viruses are not highly cytopathogenic, only occasional strains exhibiting appreciable degrees of cellular destruction; on the whole, cytopathic effects are usually absent, or so slight as to make difficult their detection. To detect the presence of virus in the culture as revealed by the presence of hemagglutinins, Vogel and Shelokov introduced the hemadsorption technique. Guinea pig or human group O erythrocytes are added to the culture tubes and the cell monolayers are then examined microscopically, using a low-power lens. A positive reaction is characterized by a firm attachment of erythrocytes to the cell sheet, with rosettes and chains of erythrocytes forming a distinctive and readily recognizable pattern. Occasionally, a positive hemadsorption test may be encountered as early as twenty-four to thirty-six hours after inoculation of the culture but, if the test is negative, the cultures should be incubated for an additional five or six days; the erythrocyte suspension originally

added is not removed and the cultures are examined at intervals for evidence of hemadsorption. However, as with the embryonated egg, several transfers in the tissue culture system may be required before hemagglutinin production reaches detectable levels or before the results can be considered negative.

Hemadsorption apparently constitutes a more sensitive method for detecting hemagglutinins and hence should afford a more rapid method for the detection (but not necessarily for typing) of influenza viruses in clinical materials, assuming that monkey kidney cell cultures and embryonated hens' eggs are equally susceptible to infection. Unfortunately, it would appear from the limited information thus far available that there is a marked difference in the susceptibility of the two systems. Kalter and associates (7) compared the relative sensitivities of the chick embryo isolation technique (amniotic inoculation) and the tissue culture hemadsorption technique by examining 102 specimens in parallel by both methods. Of these 102 specimens of throat washings or lung tissue, thirty-five were positive by one technique or the other. However, virus was recovered from only three specimens by the monkey kidney cell culture-hemadsorption method, so that this technique was definitely less sensitive in their experience. On the other hand, two of the three specimens which yielded virus by the tissue culture method failed to do so by the chick embryo technique. A recent comparison of chick embryo and monkey kidney cell culture techniques in our laboratory gave similar results; the hemadsorption technique per se was not used, the egg and the culture fluids being compared for hemagglutinative capacity by the usual method employing serologic tubes.

Each throat washing was inoculated into a group of eight eggs, eleven days old; the inoculum consisted of 0.1 ml. amniotically. The eggs were incubated for seventy-two hours for the first passage, and forty-eight hours for subsequent passages, before the fluids were harvested. Amniotic and allantoic fluids from each egg were tested against chicken erythrocytes and human group O erythrocytes, and three intra-amniotic passages were made before the results were considered negative. Throat washings were also inoculated into eight tubes of monkey kidney cell cultures, the inoculum being 0.1 ml. per tube. The tubes were examined daily for evidence of cytopathic effect over a period of eight days; at the end of the eight-day observation period, the fluid from each culture tube was distributed into Kahn serologic tubes (12 by 75 mm.) and tested for hemagglutinin content against chicken and human erythrocytes. Three passages at eight-day intervals were made before the specimen being tested was considered negative.

Eight of the sixteen specimens yielded a virus by the chick embryo method, but several passages beyond the first were required before the hemagglutinin content of the fluids was sufficiently high to permit immunologic typing. The laboratory records show that four strains were identified at the third passage level, or the seventh day; two were identified at the fourth passage level, or the ninth day; one was identified at the fifth passage level, or the eleventh day; and one was identified at the sixth passage level, or the thirteenth day. With monkey kidney cell cultures, however, only one isolate was recovered; this was typed on the seventh day and represented a specimen which was also positive on egg inoculation. The remaining fifteen specimens were carried through three passages at eight-day intervals so that an observation period of twenty-four days elapsed before the results could be considered indicative of an absence of virus in the original specimen.

While, because of the inability of many strains of virus to multiply in monkey kidney cell cultures, the hemadsorption technique may not be as useful for the isolation and identification of influenza viruses as was originally thought, the method is nevertheless an important diagnostic adjunct to the study of viral respiratory disease, e.g., for the detection and isolation of the hemadsorption viruses.

One other aspect of the diagnosis of influenza by virus recovery procedures merits some discussion, namely, the rapidity and ease of identification of the isolates. Detection of the presence of virus in the laboratory host system used has depended entirely upon detection of hemagglutinins, and subsequent classification as to immunologic type or subtype has been based upon the hemagglutination-inhibition technique. Within recent years, there has developed an increasing awareness of the difficulties and problems which surround these approaches. We have already mentioned the problem of low yields of hemagglutinin in early passages of new isolates. There is also the vexing problem of mucopolysaccharide or mucoprotein substances in serum which may bring about nonspecific inhibition of agglutination. The extent of nonspecific inhibition may be so extensive under some circum-

stances as to preclude typing of the isolate. Pretreatment of serum with filtrates from *Vibrio cholerae* cultures, which contain a receptor-destroying enzyme (RDE), or with trypsin will destroy the mucoprotein inhibitors that combine with many strains of influenza viruses. These procedures, however, have not been too helpful in destroying nonspecific inhibitors with an affinity for isolates of the Asian or A2 virus; fortunately, treatment of serum with periodate solutions has been found to destroy nonspecific inhibitors to which the so-called "animal line" strains of the Asian virus are susceptible. Differences are encountered among strains in their sensitivity to antibody, and notable changes in the behavior of the virus may occur on adaptation to new hosts (elimination of Q phase condition in the P-Q phase variation described by van der Veen and Mulder in 1950 (8)).

The complement-fixation test possesses none of these disadvantages; yet strangely enough, it has found only a limited application in the diagnosis of influenza. The type-specific soluble (S) antigen is identical for all virus strains within an immunologic type and hence it is possible through the use of specific antisera to classify fresh isolates as to type (9–11). The recent studies of Lief and Henle and their associates (4) have made it possible to obtain clean preparations of the strain-specific virus (V) antigen and of the common type-specific soluble antigen, i.e., to obtain type-specific soluble antigen free of strain-specific virus components and strain-specific virus antigen free of demonstrable amounts of type-specific soluble material. The availability of these clean antigens makes feasible the preparation of highly specific anti–S and anti–V sera; of special interest here is the fact that the anti–V sera are highly strain-specific and hence it is now possible to effect rapid identification and typing of new isolates (4). Through the use of such strain-specific anti–V sera, strains representing slightly different antigenic variants of an established old subtype, such as the A1 group, can be distinguished from each other or from a markedly different and essentially new antigenic variant, such as the A2 subtype. An important feature is the fact that the test can be done in most instances as soon as the hemagglutinins appear, even when the titers are extremely low, since in such cases compensatory increases in the volume of antigen employed are feasible. Our own experience with this method of typing is limited to several isolates during the 1960 outbreak of influenza in California, but the results have been highly gratifying and we plan to continue these studies with a view to applying this technique to the routine identification and typing of influenza viruses.

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DIAGNOSIS OF INFLUENZA BY SEROLOGIC METHODS[1]

KEITH E. JENSEN

This assignment is to cover ground that has been well-trod in years past by a majority of investigators at this conference, many of whom have been instrumental in development and evaluation of serologic methods for diagnosis of influenza. Time allotted for the presentation does not permit an historical review, and a summary of innovations and variations of procedures practiced in laboratories of the world does not appear to be warranted. Instead, the intention is to discuss data resulting from antibody measurements utilizing methods currently recommended by the United States Public Health Service and commonly employed in diagnostic centers, namely, the complement-fixation and hemagglutination-inhibition tests.

There is a general agreement that serologic diagnosis is most readily obtained using a complement-fixation system with a battery of antigens in which strains of types A and B virus are included among other agents of respiratory disease. In the midst of an epidemic when more precise definition of etiologic subgroups is required, hemagglutination-inhibition tests are carried out either secondarily to, or in conjunction with, the complement-fixation tests. Let us now consider some of the data obtained in 1957 during the time when it was of interest to use the hemagglutination-inhibition test to identify cases as due to Asian variants. Paired sera were also tested by a conventional complement-fixation method with an Asian isolate as antigen.

In figure 1, the distribution of hemagglutination-inhibition test to identify cases as due to body titers is shown for acute and convalescent sera from 41 serologically proved cases of influenza in adults. The geometric mean titers are indicated at the top of each bar. Two contrasts in the results are evident. First, while only few acute phase specimens contained hemagglutination-inhibition antibody and those were in low titer, the complement-fixation test detected a majority with antibody. During convalescence, more than 80 per cent developed complement-fixation antibody in titers of 1:32 or greater, while only 30 per cent of the group produced hemagglutination-inhibition antibody levels of 1:40 or greater. Since these were primary responses of adults to a new antigen, it was common to find less antibody by hemagglutination-inhibition than by complement-fixation tests, presumably because the latter method detected a wider spectrum of antibodies. Incidentally, the Japan/305 animal-transferred line which is sensitive to antibody and inhibitor was used throughout the studies reported here. It was shown in 1957 that hemagglutination-inhibition antibody could be more readily measured with this virus provided that serum was first treated to remove non-antibody inhibitors. The use of periodate to destroy inhibitors was adopted when receptor-destroying enzyme (RDE) and trypsin digestion were found ineffective. More recently it was found preferable to use a treatment method which combines trypsin and periodate because trypsin will digest inhibitors which combine with hemagglutinins of other type A strains and B isolates, while the periodate is effective against the Asian strain inhibitors. The current procedure is illustrated in figure 2. The total time required for treatment is approximately one and one-half hours. Serum which has been diluted by treatment to 1:7.5 is ordinarily adjusted to 1:10 with phosphate-buffered saline before making serial doubling dilutions. Appropriate controls are routinely included to ensure complete removal of inhibitors. This method has been found effective with human and all animal sera except that of chickens. Since trypsin destroys antibody in chicken serum, that step is omitted and only periodate treatment is necessary.

Some of the differences in results obtained by using the complement-fixation and hemagglutination-inhibition tests relate to the interval between collection of onset and convalescent serum specimens, as depicted in figure 3. From the examination of 422 serologically proved cases, it may be seen that the complement-fixation test is much superior to the hemagglutination-inhibition test when the second serum sample is obtained from one to two weeks after the acute-phase specimen, but that the margin decreases as the interval is increased. Occasionally, however, some of the cases diagnosed by the complement-fixation test

[1] From the Biologics Research Department, Chas. Pfizer & Co., Inc., Terre Haute, Indiana.

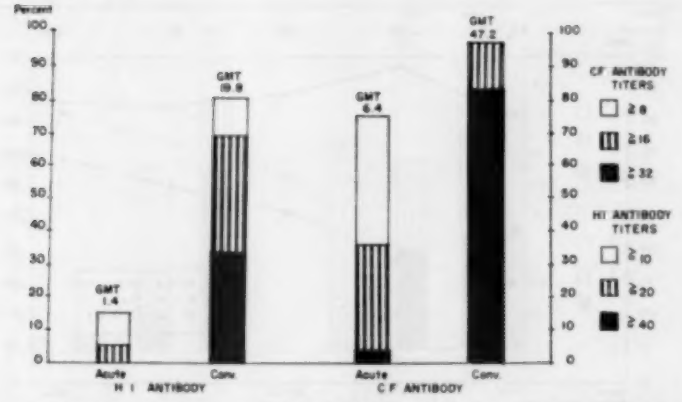

FIG. 1. Distribution of hemagglutination-inhibition (HI) or complement-fixation (CF) antibody titers in acute and convalescent sera from 41 proved cases of influenza in adults.

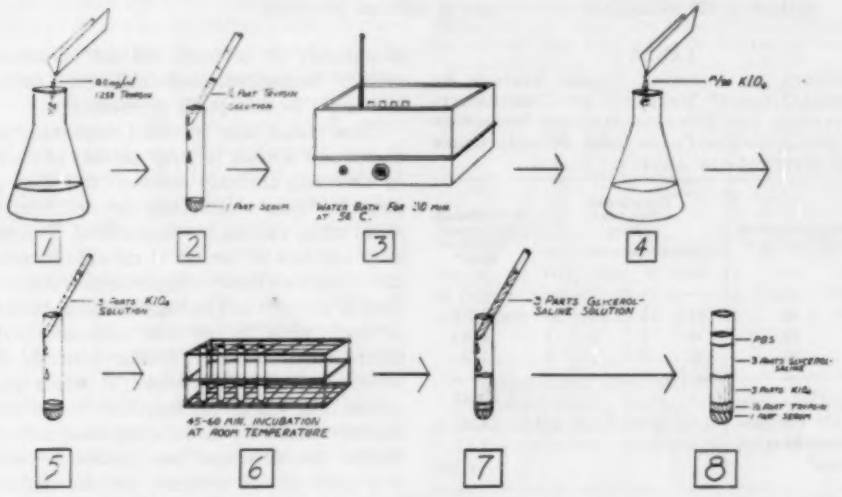

FIG. 2. Trypsin-KIO_4 treatment for removal of nonspecific inhibitors from influenza serum.

are missed by hemagglutination-inhibition, while a few will be detected by the hemagglutination-inhibition method which are not found by complement-fixation. When nineteen days or more elapsed between collection of acute and convalescent serum specimens, 70 per cent were diagnosed with both the complement-fixation and hemagglutination-inhibition methods, while 82 per cent were found with the hemagglutination-inhibition test, and 87 per cent with the complement-fixation test. Therefore, two conclusions seem warranted: (*1*) When maximal efficiency is required it is necessary to employ both tests; (*2*) examination of a second convalescent serum specimen may yield results not obtained with a sample taken early in the course of recovery from illness.

Another aspect of this problem is apparent when comparing the distribution of antibody titers in acute-phase sera as measured by the two methods. Data from a series of 41 cases are shown in table 1. There were 35 in which acute-phase

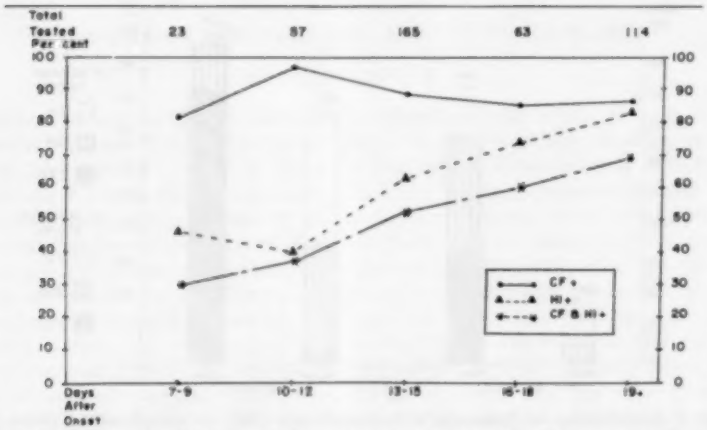

FIG. 3. The influence of interval between onset and convalescent serum specimens on efficiency of diagnosis by complement-fixation (CF) and hemagglutination-inhibition (HI) methods in 422 serologically proved cases of influenza infections.

TABLE 1

INCIDENCE OF ANTIBODY TITERS AGAINST A/ASIAN/JAPAN/57 MEASURED BY COMPLEMENT-FIXATION AND HEMAGGLUTINATION-INHIBITION TESTS IN ACUTE-PHASE SERA FROM 41 CASES OF INFLUENZA IN ADULTS

Hemagglutination-Inhibition Titers	Complement-Fixation Titers				Total Hemagglutination-Inhibition Rises*
	<8	8	16	32	
<10	10	15	10	0	22/35
10	0	1	2	1	4/4
20	0	0	1	1	1/2
Total complement-fixation rises*	9/10	15/16	7/13	2/2	27/41 33/41

* Fourfold titer increases in convalescent sera.

hemagglutination-inhibition titers were <1:10. Only 22 of those patients developed the significant fourfold increases by hemagglutination-inhibition while all responded as measured by complement-fixation tests. Conversely, 8 cases did not produce diagnostic complement-fixation rises, and 6 of these were in the group which had acute-phase complement-fixation titers of 1:16. Therefore, approximately 20 per cent did not respond to infection in a manner detectable by complement-fixation tests. More importantly perhaps, these and other data suggest that approximately 20 per cent did not manufacture specific hemagglutination-inhibition antibody and might be susceptible to reinfection.

These results take on added significance when an attempt is made to judge potency of vaccines by observing antibody responses in human subjects. In figure 4 data from the first trial with Asian strain vaccine are summarized. Two weeks after one dose of vaccine (1 ml. subcutaneously, 200 chicken erythrocyte agglutination units), less than 50 per cent had hemagglutination-inhibition antibody while 90 per cent contained complement-fixation antibody. Similar antibody titers were found after four weeks, at which time a second dose was given. Responses to the second injection were very encouraging when more than 80 per cent developed hemagglutination-inhibition antibody. In contrast, the distribution of antibody titers measured by the complement-fixation test was not much changed as a result of the second dose.

Since a portion of the study group did not develop antibody as a result of vaccination, and infection did not always stimulate antibody, the question arises as to how efficient serologic methods are, particularly in vaccinated populations, in defining cases of influenza. An example of the problem is stated in table 2. The data are from a study carried out at a penitentiary in Georgia where groups of 300 prisoners were given vaccines of varied potency or a placebo. As expected, and not shown here, about 50 per cent developed

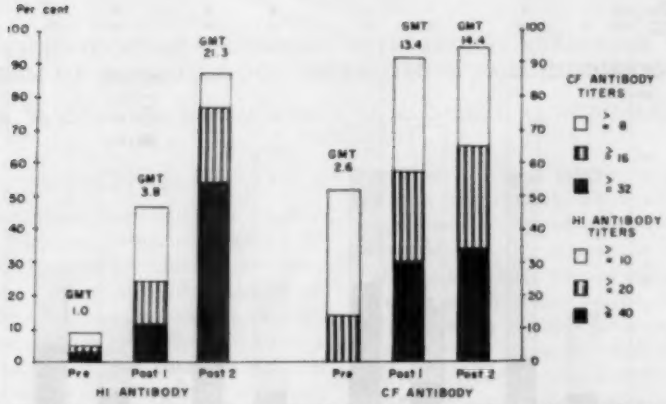

FIG. 4. Distribution of hemagglutination-inhibition (HI) and complement-fixation (CF) antibody titers before and after one and two doses of Asian influenza vaccine in 59 adults.

hemagglutination-inhibition antibody after the one dose of 50 chicken erythrocyte agglutination unit vaccine and about 90 per cent responded to the most potent vaccine. Six weeks after vaccination, the fall epidemic began with cases of acute febrile respiratory illnesses from which Asian virus was isolated repeatedly. Ten per cent of the afebrile cases could be defined serologically as influenza, and only slight correlation was found with antibody status in the onset serum. On the other hand, patients with a fever developed fourfold or greater antibody rises with a frequency which varied with the incidence of acute-phase hemagglutination-inhibition antibody. Of course, conclusions from such a limited study may not be sound, and some of the febrile illness may have been due to unknown etiology; however, it appears that serologic diagnosis is only about 80 per cent efficient in an adult population without previous hemagglutination-inhibition antibody. In the group in which many had antibody as a result of vaccination, fewer cases could be diagnosed serologically as influenza. Either these were not influenzal infections or serum antibody may interfere with the usual or normal immunologic response to antigenic stimuli of infection.

This dilemma is a well-known one and, in the past, attempts have been made to differentiate response to vaccination from that of convalescence by using the complement-fixation test with soluble antigen such as that extracted from membranes of infected embryos. This approach is no longer useful, however, since it is now evident

TABLE 2

EFFICIENCY OF SEROLOGIC DIAGNOSIS AS RELATED TO FEVER AND ACUTE SERUM ANTIBODY TITER

Vaccine Group	Afebrile*			Febrile		
	Number Tested	Acute Hemagglutination-Inhibition Titer < 20	Serologic Diagnosis†	Number Tested	Acute Hemagglutination-Inhibition Titer < 20	Serologic Diagnosis
		per cent	*per cent*		*per cent*	*per cent*
Placebo	30	100	10	19	100	89
50 CCA‡	38	59	10	12	100	75
200 or 500 CCA	67	35	6	11	64	36

* Temperatures never at 100°F.

† Fourfold rise by hemagglutination-inhibition or complement-fixation.

‡ CCA—chicken erythrocyte agglutination units.

that current vaccines (produced by centrifugation rather than elution from erythroctyes) often contain soluble antigen. Data supporting this conclusion are charted in figure 5. Adults injected with the Asian vaccine at two different potencies produced antibody patterns which were very similar when measured with the Japan/305 viral or membrane or PR8 viral. In addition, it has been found by Henle and his associates that children without previous influenzal experience often do not develop anti-soluble antibodies as a result of infection.

Thus, we have seen that, under the immunologic

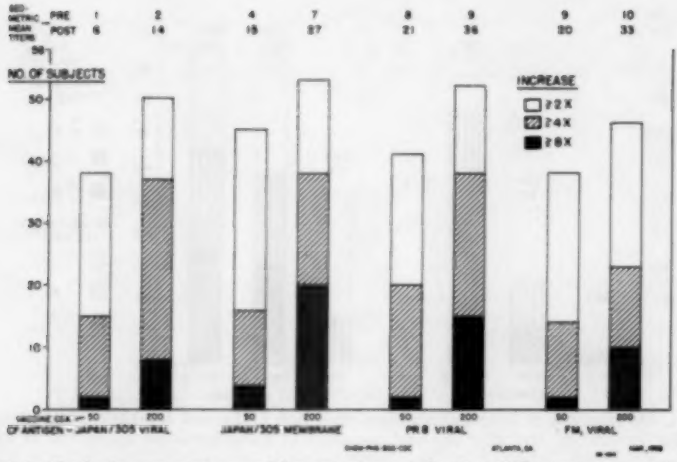

FIG. 5. Antibody increases measured by complement-fixation (CF) tests in sera from 58 adults injected subcutaneously with Lot 1 of Asian vaccine.

conditions prevailing during the 1957 pandemic, a combination of complement-fixation and hemagglutination-inhibition tests could detect at least 80 per cent of cases in adults. It will be interesting to learn whether comparable data were obtained in 1960 when many patients might have had previous experience with this antigenic mosaic and therefore could be expected to respond in a different manner. As the newer methods for laboratory diagnosis are developed, perhaps it will become possible to define cases with greater speed and accuracy, although no other methods have yet appeared as suitable for large-scale surveys as are the serologic tests.

THE HEMADSORPTION TECHNIQUE, WITH SPECIAL REFERENCE TO THE PROBLEM OF NATURALLY OCCURRING SIMIAN PARA-INFLUENZA VIRUS[1]

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Hemadsorption

The phenomenon of hemadsorption was first described by Vogel and Shelokov in 1957 (1, 2). This technique has greatly facilitated the recovery of influenza virus and its study in the laboratory. In addition, hemadsorption was essential to the discovery of the parainfluenza viruses, a group of agents which grow poorly in eggs and produce little or no cytopathogenic effect during primary passage in tissue culture (3–5).

Hemadsorption occurs when erythrocytes added to a myxovirus-infected tissue culture are adsorbed to the host-cell surface. The erythrocytes combine with virus-specific material at the cell surface, since the reaction can be prevented by prior treatment of the tissue culture with immune serum. The combining sites on the erythrocyte are apparently similar to those involved in the hemagglutination reaction, for receptor-destroying-enzyme (RDE) treatment of erythrocytes results in a loss of their capacity to be hemadsorbed. Thus, hemadsorption, like hemagglutination, appears to involve binding between virus outer-coat material and erythrocyte mucoprotein receptor sites.

If current concepts of influenza virus replication are correct, then several factors in myxovirus maturation and release would seem to favor the occurrence of hemadsorption. Final assembly of the infectious particle from the genetic and outer-coat materials is thought to occur at or near the host-cell surface with virus release proceeding as a process of extrusion; in some instances filament formation is involved (6). Release of virus from cells is known to occur over a long period of time (7). Virus-specific material is thus present at the cell surface for a prolonged period, providing sites to which erythrocytes can adsorb. It is of some interest that hemadsorption also occurs in cultures infected with vaccinia, a virus which is slowly released from infected cells over a long period of time (8). The ability of a virus to hemagglutinate certain erythrocytes is not always associated with the capacity of these erythrocytes to adsorb onto infected cells in cultures, as shown in table 3. It may well be, as suggested by the information available for some of the agents exhibiting this disparity, that the failure to hemadsorb reflects a more abrupt type of release mechanism, virus being present at the cell surface for short periods of time.

As shown in table 3, primary cultures infected with any of the myxoviruses are capable of hemadsorbing guinea pig erythrocytes. Employing this technique, strains of influenza A2; B; parainfluenza 1, 2, 3, and 4; and mumps have been recovered from patients with illness (2–4, 9).

As a standard method in virus isolation studies, we have employed guinea pig erythrocytes at a concentration of 0.4 per cent. Two-tenths of a milliliter of such a suspension is added to a culture tube which contains 1 ml. of fluid medium. Incubation of the cultures to which erythrocytes have been added is usually carried out for thirty minutes at 4°C. All of the myxovirus-infected cultures give a strong hemadsorption reaction with guinea pig erythrocytes at this temperature, except parainfluenza 4 virus which requires 25° to 37°C. for maximal patterns. Temperatures above 25°C. cause a reversal of positive patterns in cultures infected with parainfluenza 1 and 3 viruses.

Tissue cultures inoculated with specimens from individuals with illness are tested for hemadsorption after a five-day incubation interval and subsequently at the tenth to thirteenth and twentieth days. The quantity of free virus in the fluid medium is usually insufficient to interfere with the hemadsorption reaction. During experimental laboratory studies, however, quantities of free virus, sufficient to interfere with hemadsorption, are found in the medium three to five days following inoculation of cultures with 10^4 to 10^6 TCD 50 of influenza or parainfluenza virus. Because of the formation of large aggregates of agglutinated erythrocytes in the medium, few such cells are available for hemadsorption. It is advisable when large quantities of virus have been inoculated, to wash the cultures three times before attempting to test for hemadsorption.

The time required for detection of various myxoviruses in monkey kidney tissue culture is

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TABLE 3

HEMAGGLUTINATING VIRUSES ASSAYED FOR HEMADSORPTION WITH ERYTHROCYTES REACTIVE IN HEMAGGLUTINATION TEST

Virus	Tissue Culture	Result of Hemadsorption with Indicated Erythrocytes	
		Positive	Negative
Influenza A2*	Monkey kidney	Guinea pig, chick, sheep, human O, rhesus monkey	
Influenza B	Monkey kidney	Guinea pig, rhesus monkey	
Influenza C	Monkey kidney	Guinea pig	
Parainfluenza 1	Monkey kidney, human kidney	Guinea pig, rhesus monkey	
Parainfluenza 2	Monkey kidney	Guinea pig, rhesus monkey	
Parainfluenza 3	Monkey kidney, human kidney	Guinea pig, rhesus monkey	
Parainfluenza 4	Monkey kidney, human kidney	Guinea pig, rhesus monkey	
Mumps*	Monkey kidney, human kidney	Guinea pig, rhesus monkey	
NDV*	Monkey kidney	Guinea pig, rhesus monkey	
Vaccinia*	Monkey kidney	Chicken	
ECHO 3, 6, 7, 11, 19*· †	Monkey kidney		Human O
Reovirus, (Type 1)†	Monkey kidney		Human O
Polyoma‡	Mouse embryo		Guinea pig

* Data of Shelokov *et al.*
† Data of L. Rosen.
‡ Data of W. Rowe and J. Hartley.

TABLE 4

RECOVERY OF VARIOUS MYXOVIRUSES FROM THROAT SWAB SPECIMENS: TIME REQUIRED FOR DETECTION BY HEMADSORPTION IN MONKEY KIDNEY TISSUE CULTURE

Virus	Total Isolations	Per Cent Detected in Indicated Interval (Days)		
		5	10–13	18 or >
Parainfluenza 1	185	82.7	12.4	4.9
Parainfluenza 3	447	88.1	8.5	3.4
Parainfluenza 4	35	11.4	71.4	17.2
Mumps	36	72.2	22.2	5.6
Influenza B/MD/59	34	85.3	14.7	0
Influenza A2/MD/58	43	95.3	4.7	0

shown in table 4. The majority of isolates of all but parainfluenza 4 virus were detected by the fifth day. Shelokov and associates have reported that influenza A2 can be recognized by hemadsorption within forty hours (1). These workers have also presented data which indicated that growth of influenza A2 in monkey kidney culture is optimal at pH 6.5 and inhibited by an alkaline pH of 8.0. The presence of influenza or other myxoviruses can usually be detected by hemadsorption one to two days prior to the occurrence of cytopathic effect. Certain naturally occurring strains of influenza A2 and B produce little or no cytopathic effect during primary passage in tissue culture and would probably be missed without the aid of the hemadsorption technique. The parainfluenza viruses characteristically produce no effects or minimal visible effects in culture during primary passage, and the type 1 (hemadsorption type 2) and type 4 strains recovered in this country characteristically fail to produce an obvious cytopathic change upon serial tissue culture passage. In these instances we must rely completely upon the hemadsorption reaction.

The sensitivity of tissue culture combined with hemadsorption in the isolation of influenza A2 was compared with that of embryonated eggs by Shelokov and co-workers (2). As shown in table 5, in which their findings are summarized, monkey kidney culture was found to be more sensi-

TABLE 5

COMPARISON OF EMBRYONATED EGG AND MONKEY KIDNEY TISSUE CULTURE HEMADSORPTION TECHNIQUES FOR ISOLATION OF INFLUENZA A2 VIRUS*

Number of Specimens Tested (Three Outbreaks)	Virus Recovery		
	Egg	Tissue Culture Hemadsorption	Total
93	10	21	22

* Data from Shelokov *et al.* (2)

tive than the hen's egg. Other workers, however, have reported that the embryonated egg was more sensitive, but it is not clear whether the factors of an acid- and serum-free medium were controlled in these studies (10, 11). It may be that strains of influenza A2 in nature vary in their capacity for growth in eggs and tissue culture.

Although we have not compared the sensitivities of eggs and tissue culture, it is our impression that the latter system is a relatively effective method for recovery of influenza A2. During the "second wave" of influenza A2 in Washington in February and March, 1958, this agent was recovered in monkey kidney culture from 15 per cent of 137 children with upper respiratory illness.

The efficacy of monkey kidney and eggs for isolation of influenza B was studied by Takemoto and colleagues (12). From seventy-eight specimens tested, seventeen strains were recovered in tissue culture and only one in eggs.

Monkey kidney tissue culture combined with hemadsorption offers several advantages over the embryonated egg in the study of influenza and other myxoviruses. Tissue cultures can be held for one to two weeks before being employed for virus isolation, whereas eggs must be used when they are of a certain age. In addition, monkey kidney permits the recovery of certain other agents which grow poorly in eggs and which are associated with febrile respiratory illness clinically indistinguishable from influenza. Important in this regard are the naturally occurring strains of the parainfluenza viruses found in the United States and Europe (3, 4, 13, 14).

Occurrence of a Simian Para-influenza Virus in Monkey Kidney Tissue Culture

The use of monkey kidney tissue culture for the recovery of myxoviruses does involve one serious disadvantage. This is the frequent natural occurrence of a myxovirus of simian origin in the tissue culture system. This agent has all the properties of a parainfluenza virus and is identical with the SV-5 virus of Hull (15).

The simian hemadsorption agent has a size of 90 to 150 μ by gradocol filtration, is ether-sensitive, and agglutinates chicken erythrocytes to a high titer. The agent multiplies in both the amniotic and allantoic cavity of the embryonated hen's egg with the production of hemagglutinins.

TABLE 6
RELATION OF SIMIAN HEMADSORPTION AND PARAINFLUENZA 2 (CA) VIRUSES

Guinea Pig Serum	Reciprocal of Antibody Titer with Four Units Indicated Antigen			
	Complement Fixation		Hemagglutination-Inhibition	
	Simian	Para-Influenza 2		
	Allantoic Fluid	Monkey Kidney Tissue Culture Fluid	Simian	Para-influenza 2
Simian hemadsorption:				
Pre-immunization	<10	<10	<10	<10
Post-immunization	320	80	1280	<10
Parainfluenza 2:				
Pre-immunization	<10	<10	<10	<10
Post-immunization	<10	320	<10	640

The receptor sites on the erythrocytes are sensitive to the action of receptor-destroying enzyme.

Extensive serologic testing has shown that the simian virus is antigenically distinct from any of the known myxoviruses with the exception of parainfluenza 2 (CA) virus. The relationship to parainfluenza 2 virus is shown in table 6. A one-way antigenic cross relation exists in which simian virus reacts by complement-fixation with both its homologous serum and that prepared against parainfluenza 2 virus. These agents were distinct in the hemagglutination-inhibition test.

One of four adults infected with parainfluenza 3 virus and one of four persons infected with mumps virus developed a complement-fixation antibody rise for the simian virus. These findings are consistent with the concept that the simian virus belongs to the parainfluenza group, since heterologous rises among these agents are commonly observed, especially among adults. Henceforth, we shall refer to this agent as the simian parainfluenza virus.

Fifteen of eighty-five lots of monkey kidney cultures were found to contain this agent over a thirteen-month period. The agent had a definite seasonal distribution, occurring only from October through March. The extent of contamination of different lots of monkey kidney cultures varied from 0.5 to 90 per cent. It has been our policy to include twenty uninoculated cultures in each isolation test for every one hundred tubes

inoculated with specimens. In most instances, employing this ratio, it has been possible to detect simian parainfluenza virus in the uninoculated controls when it has appeared in the inoculated cultures. However, when the level of contamination was as low as 0.5 to 1 per cent, recovery from control cultures was not always achieved. The failure of individuals whose inoculated cultures yielded the agent to develop an antibody rise suggests that the "isolates" were of simian origin.

The simian parainfluenza virus has been detected in different regions of the United States and in Great Britain. It is identical with the SA virus described by Schultz and Habel (16).

We have been unable to obtain evidence that the simian para-influenza virus infects man. Two hundred paired sera from adults with respiratory illness were tested, but no antibody rises were detected. Complement-fixing antibody for the simian parainfluenza virus was observed, but it correlated with the presence of antibody for parainfluenza 2 or 3 viruses. The simian parainfluenza virus was not recovered from 655 children and adults with respiratory illness whose pharyngeal swabs were tested in human embryonic kidney cultures, a system which is extremely sensitive for this agent. Seventeen parainfluenza viruses were recovered, but they were all of the known human types. It must be emphasized that these findings do not rule out the possibility that this agent infects man infrequently.

During the past fifteen months we have been able to control simian parainfluenza virus contamination by adding 0.2 per cent hyperimmune rabbit serum to both the growth and maintenance media of monkey kidney cultures. The agent has been detected only once during this interval although large lots of 1,400 cultures were used each week. Simian immune serum in the concentration employed did not inhibit parainfluenza 2 virus. Nevertheless, there is cause for concern. There may well be undetected human hemadsorption agents which share major antigens with the simian virus. If so, they will not be recovered when cultures containing simian immune serum are employed. The presence of this agent in monkey kidneys also offers a major obstacle to the use of such tissue for production of live virus vaccines. Information concerning the natural history of simian hemadsorption virus is urgently needed.

Addendum

Since this manuscript was submitted for publication, Emory and York (Virology, 1960, *11*, 313) have reported the recovery of several strains of a hemadsorption virus from normal monkey kidney tissue cultures. They described a serologic relationship of these strains with parainfluenza virus. Analysis of their materials in this laboratory revealed that the viruses were all similar to those described in this paper and that their parainfluenza antiserum was actually simian parainfluenza serum. This finding accounts for the discrepancy between their data and those reported in the communication.

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DIAGNOSIS OF INFLUENZAL INFECTION BY MEANS OF FLUORESCENT ANTIBODY STAINING[1]

CHIEN LIU

To establish the diagnosis of influenzal infection either by serologic reactions or by isolation of virus is time consuming. Rarely can the results of laboratory diagnosis be completed while the patient is still sick. The fluorescent antibody staining technique as developed by Coons and associates (1, 2), by virtue of its sensitivity and immunologic specificity, offers a new approach for rapid diagnosis of influenzal infection.

Watson and Coons (3) first demonstrated influenza virus antigens by fluorescent antibody staining in the infected cells lining the amnion and in the epidermal and pharyngeal epithelium of chick embryos. In ferrets experimentally infected by intranasal inoculation, Liu (4) was able to localize the site of influenza virus multiplication exclusively in the epithelial cells lining the nasal turbinates and the bronchi. During the period of virus multiplication, degeneration and desquamation of the respiratory epithelium occurred which had been described previously by Francis and Stuart-Harris (5) and also by Perrin and Oliphant (6).

During the febrile period of the ferrets' illness, fluorescent cells containing specific influenza virus antigens were found in the nasal discharge, the presence of which allowed a rapid diagnosis of influenzal infection. These specific fluorescent cells were found during the febrile period in the infected animals (table 7). However, in one ferret in which no apparent clinical signs of influenzal infection were noticeable, positive nasal smears obtained by fluorescent staining established the diagnosis of infection, which subsequently was confirmed by rise of antibody.

With this background, the technique was applied to the diagnosis of human influenzal infections (7). During an A1 epidemic, the diagnosis was established by finding cells containing influenza antigen in nasal smears in twelve of seventeen patients (71 per cent), confirmed as having influenzal infection either by virus isolation or by a fourfold or greater rise of hemagglutination-inhibition antibody titer (table 8).

[1] From the Department of Pediatrics and the Hixon Memorial Laboratory, University of Kansas School of Medicine, Kansas City, Kansas.

During an influenza B epidemic, only ten of twenty-six cases (38 per cent) were positive by fluorescent staining. The results obtained during the 1957 Asian influenza epidemic were similar. Among fourteen patients with influenza-like illnesses, nine showed positive nasal smears when stained with either anti-PR8 or anti-Malaya/311/57 fluorescent conjugate. All smears were negative when stained with the anti-Lee B conjugate. In these nine positive cases, only two patients showed a significant fourfold or greater rise of hemagglutination-inhibition antibody against homologous virus during convalescence, while five of them, including the above two patients, showed a significant rise of complement-fixing antibody against type-specific soluble antigen. During the same 1957 Asian epidemic, Silverstein, Cheatham, and Eveland (8) at the Armed Forces Institute of Pathology also used the fluorescent staining method to examine nasal smears from ten cases of influenza-like illness (table 9). They obtained positive smears in seven cases. From six of these seven patients, a hemagglutinating agent was isolated; three of these agents were subsequently confirmed to be Asian influenza virus. The author is grateful to Dr. Silverstein and his associates who have allowed us to include these unpublished data in this report. As a control study, one false-positive case by fluorescent staining was encountered among twenty-three cases of non-influenzal upper respiratory infections (7).

In addition to the examination of nasal smears by fluorescent antibody staining, the technique was also applied to the study of frozen sections from autopsied specimens in thirty-two fatal Asian influenza cases. Martin and associates (9) reported that seven of twenty-nine lungs and five of nineteen tracheas examined had influenza virus antigen in the macrophages scattered in the tissues. These positive fluorescent staining specimens were all confirmed either by positive isolation of virus from such organs or by the presence of antibody in the patients' sera. This indicates that fluorescent antibody staining may be a useful adjunct for post-mortem examinations.

Kirber and Henle (10) have reported that the

TABLE 7

RELATION OF FEVER TO THE PRESENCE OF FLUORESCENT CELLS IN NASAL SMEARS OF FERRETS INOCULATED WITH INFLUENZA A VIRUS

Ferret Number	Time after Inoculation	Temperature	Nasal Smears	Virus Titration	
				Nasal mucosa	Lung
	days	°F		Log EID_{50}	Log EID_{50}
V 2-58 (Far.)	1	101.4	0	2.5	4.5
V 2-59 (Far.)	1	101.4	0		
	2	102.4	0	4.5	0.5
V 2-60 (Far.)	1	101.0	0		
	2	104.2	++*		
	3	104.6	+++	5.3	1.5
V 2-61 (Far.)	1	102.0	0		
	2	101.6	0		
	3	105.4	+++		
	4	101.4	±	5.5	4.5
V 4-52 (PR8)	1	101.5	0		
	2	101.9	±		
	3	102.0	+++		
	4	99.9	++++		
	5	100.4	+++		
	7	101.3	±		
	8	100.4	0		
	9	101.0	0	<1.5	<1.5†

* Number of fluorescent cells in smears: +++, numerous; ++, moderate; +, few; ±, doubtful.

† Serum hemagglutination-inhibition antibody titer against PR8 virus: pre-inoculation <1/5; on ninth day >1/2560.

(*Reproduced with permission from the Journal of Experimental Medicine, 1955, 101, 665.*)

S antigen occurred as a common antigen among all strains of influenza virus belonging to the same type. This S antigen was apparently responsible for the cross reactions in fluorescent staining. The S antigen was primarily localized in the nucleus, although to a lesser extent it was also found in the cytoplasm (11). Breitenfeld and Schäfer (12) have shown that the G antigen of fowl plague virus, which is apparently equivalent to the soluble antigen in influenza, appeared in the nucleus of infected cells prior to its detection in the cytoplasm.

Since both the Asian virus and the PR8 virus are type A viruses, it was anticipated that there would be cross reactions between them by fluorescent staining. This was demonstrated by treating the frozen sections from Asian and PR8 infected chorioallantoic membranes with homologous and heterologous conjugates (table 10). However, when the conjugates were absorbed with the soluble S antigen prepared from PR8 virus, staining on heterologous tissues was completely abolished while only the nuclear staining on homologous tissues was affected, leaving the cytoplasmic fluorescence representing the V antigen in the cytoplasm. Absorption of the conjugates with the S antigen from influenza B virus (Lee strain) had no effect on the nuclear fluorescence. From these data it was evident that a strain-specific conjugate could be prepared for staining influenza-infected tissues.

When nasal smears from Asian influenza pa-

TABLE 8

DIAGNOSIS OF HUMAN INFLUENZA BY FLUORESCENT ANTIBODY STAINING OF NASAL SMEARS

Year	Type	Number of Confirmed* Cases	Positive Smears	Per Cent Positive
1952	A1	17	12	71
1955	B	26	10	38
1957	Asian	14†	9‡	—

* Confirmed either by virus isolation and/or fourfold or greater rise of homologous hemagglutination-inhibition antibody in convalescent sera.

† Influenza-like illnesses.

‡ Five cases had fourfold or greater rise of complement-fixing antibody against type A soluble antigen prepared from PR8 virus. Only 2 of these had a fourfold or greater rise of hemagglutination-inhibition antibody against A/Japan/305/57 virus.

TABLE 9

DIAGNOSIS OF ASIAN INFLUENZA BY FLUORESCENT ANTIBODY STAINING*

Case Number	Positive Nasal Smears	Recovery of Hemagglutination-Inhibition Agent	Asian Influenza Isolated
1	+	+	+
2	+	+	+
3	+	+	+
4	+	+	Not done
5	+	+	Not done
6	0	+	+
7	+	+	Not done
8	0	+	Not done
9	+	0	Not done
10	0	0	Not done

* Data from Silverstein, Cheatham, and Eveland (8).

TABLE 10

HOMOLOGOUS AND HETEROLOGOUS FLUORESCENT STAINING ON PR8 AND MALAYA/311/57 VIRUS INFECTED CHORIOALLANTOIC MEMBRANES

Conjugate	Site of Fluorescence	Degree of Staining in Chorioallantoic Membranes Infected with	
		PR8	Malaya
Anti-PR8 (unabsorbed)	Nucleus	+++*	+++
	Cytoplasm	+++	+
Anti-PR8 (absorbed with Lee S)	Nucleus	+++	+++
	Cytoplasm	+++	+
Anti-PR8 (absorbed with PR8 S)	Nucleus	0	0
	Cytoplasm	++	0
Anti-Malaya (unabsorbed)	Nucleus	+++	+++
	Cytoplasm	0	+++
Anti-Malaya (absorbed with Lee S)	Nucleus	+++	+++
	Cytoplasm	0	+++
Anti-Malaya (absorbed with PR8 S)	Nucleus	0	0
	Cytoplasm	0	++

* +++: Maximal fluorescence
++: Moderate fluorescence
+: Minimal fluorescence

tients, showing many virus-containing cells, as demonstrated by staining with both anti-PR8 to anti-Asian conjugates, were treated with the same anti-PR8 and anti-Asian conjugates absorbed with the PR8 S antigen, the smears became completely negative. Similar results were obtained by treating positive nasal smears from ferrets infected with Asian virus. Therefore, it was concluded that, although strain-specific fluorescent staining conjugates could be prepared by absorbing them with type-specific soluble antigen, these absorbed conjugates could only be used on infected tissue sections such as the chorioallantoic membranes or the ferret nasal turbinates. They were not useful in staining human nasal smears for the rapid diagnosis of influenza. Since the fluorescent cells in the human nasal smears were primarily macrophages, it was speculated that they contained the S antigen which rendered them stainable by fluorescent conjugates prepared from any strains of influenza virus of the same type. Once the conjugates were absorbed by the S antigen, the S antibody was removed and the conjugate became ineffective. In this respect the diagnosis of influenza infection by fluorescent staining can only be type-specific to the same extent as is the complement-fixation test employing the S antigen.

In summary, a rapid diagnosis of influenzal infections can be made by means of fluorescent antibody staining. Although the sensitivity of this method is at present lower than the conventional serologic tests or the isolation of viruses, type-specific identification by fluorescent staining can be obtained readily during the same day in which nasal washings are collected. Apparently, it is the type-specific S antigen present in the macrophages of nasal smears which gives the positive fluorescent staining. Therefore, only a type-specific rather than a strain-specific diagnosis can be obtained, and conjugates with good S antibody prepared by any influenza virus of the same type should be satisfactory for use in identification.

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General Discussion

Dr. William J. Mogabgab (New Orleans, Louisiana): The variety of procedures that may be used for the diagnosis of influenza provides the means whereby any laboratory engaged in serologic determinations or virus studies can readily participate in the detection of this disease. Refinements in technique that allow isolation of virus from the greatest proportion of infected persons or serologic procedures that reveal the antigenic subtype are of much value for special problems. Nevertheless, lack of facilities or materials for optimal results should not be allowed to deter any laboratory from applying its available tools in order to confirm the presence of influenza. Much more information on the frequency of complications could easily be acquired by assistance from general clinical and other laboratories, and localized or unexpected outbreaks could be quickly documented. Either the embryonated egg or primary tissue cultures prepared from the kidneys or lungs of monkeys or other species may be used for isolation of influenza viruses. Differences in sensitivity will result in a greater proportion of influenza A isolations in the egg than in monkey kidney cultures, but the converse will occur with influenza B.[1, 2] Even the allantoic sac of the embryonated egg, as noted by Kilbourne,[3] was suitable for recovery of Asian virus. Nevertheless, the amniotic sac of the chick embryo, by virtue of its proved capability in isolation of all types and strains of influenza viruses, should still be considered the universal method of choice for unidentified material. Kidney tissue cultures from a variety of animals provide a satisfactory system for workers not acquainted with the embryonated egg.[4] Prior to inoculation, cultures must be washed free of inhibitory animal sera and the pH should be maintained near neutrality, but second passages are not necessary if fluids are replaced in two or three days in order to remove any neutralizing substances that are sometimes contained in the inoculated respiratory secretions.[5] Hemadsorption may be used for detection of virus, but infected cultures will produce sufficient hemagglutinating antigen for identification and typing.[1, 2, 6] Evaluations of the hemadsorption technique after short periods of incubation or with cultures prepared from cell lines should also take into account that nasopharyngeal washings sometimes contain sufficient virus to agglutinate erythrocytes or to allow hemadsorption without growth of virus. Because of the variations in amount of infectious virus contained in individual nasopharyngeal washings from influenza cases, comparisons of efficacy of isolation procedures must be based upon duplicate titrations of infectious virus in each system.[1, 6] Obviously, specimens collected properly early in the disease will contain more infectious virus. Isolations from these specimens could probably be made in any of the available systems, while another group of pharyngeal washings might contain infectious virus in an amount that was below the threshold of infectivity in one system and above it in another. Nevertheless, it has been possible to isolate influenza A and B in kidney tissue cultures frequently enough to be able to identify strains causing outbreaks since 1952. Perhaps more potential for widespread application of definitive diagnosis rests with the fluorescent antibody technique, as described by Liu. Difficulties might be encountered in identification of the subtype with this procedure, but it should prove extremely useful for immediate detection of infection and surveys of incidence.

In table 11 a comparison is shown of the results of simultaneous isolation attempts in the amniotic sac and monkey kidney cultures with garglings obtained from serologically diagnosed cases of Asian influenza. The proportion of cases from which virus could be isolated in the egg was 62 per cent, but was as much as 80 to 90 per cent when specimens were carefully collected early in the illness. Infectivity titrations demonstrated that primary isolation of this strain in tissue culture required ten to one hundred times as much infectious virus as in the chick embryo. The converse had been found with the 1954 strain of influenza B.[1]

Further comparison of these two isolation systems is shown in table 12, and it should be noted that even one passage in the egg yielded more positive results than tissue culture. Occasional egg

[1] Mogabgab, W. J., *et al.*: Proc Soc Exp Biol Med, 1955, *89*, 654.

[2] Mogabgab, W. J., *et al.*: Proc Soc Exp Biol Med, 1958, *99*, 116.

[3] Kilbourne, E. D.: J Clin Invest, 1959, *38*, 266.

[4] Green, I. J., *et al.*: J Immun, 1957, *78*, 233.

[5] Shelokov, A., *et al.*: Proc Soc Exp Biol Med, 1958, *97*, 802.

[6] Mogabgab, W. J., *et al.*: J Immun, 1956, *76*, 314.

TABLE 11

VIRUS ISOLATIONS IN EGG AND IN TISSUE CULTURE FROM SPECIMENS OBTAINED FROM SEROLOGICALLY DIAGNOSED CASES*

Isolation System	Number of Cases	Number of Isolations	Per Cent of Isolations
Egg	100	62	62.0
Tissue culture (seven-day)	64	10	15.6

* Fourfold or greater increments in hemagglutination-inhibition were obtained in paired sera using the monkey kidney line of Asian virus and receptor-destroying enzyme–treated sera.

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TABLE 12

RESULTS OF INOCULATION OF EGG-POSITIVE SPECIMENS IN EGG AND IN MONKEY KIDNEY CULTURES

Isolation System	Number of Specimens	Number Positive	Per Cent Positive
Egg: E_1		33	43.4
E_2		37	48.7
$Q(E_3–E_7)$		6	7.9
Total	76	76	100.0
Tissue culture (seven-day)†	51	10	19.6
Tissue culture (HaAds)‡	27§	3	11.1

* More than two amniotic passages (E_1 and E_2) were done if hemagglutinins were not inhibited by specific antibody (Q phase).

† Fluids were replaced at three and seven days and hemagglutinating titers determined.

‡ Human O erythrocytes were added at one to two days for hemagglutination-adsorption.

§ Nine were also positive in tissue culture (seven-day).

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isolates were in the nonreactive Q phase until several passages were made, but this was not encountered in tissue culture. Rapid isolation in one to two days as determined by hemadsorption in monkey kidney cultures was the least effective method.

Serologic diagnosis of influenza in the laboratory that is uninitiated in hemagglutination-inhibition may present some difficulties. Many are quite familiar with complement-fixation, and once the strain has been identified and characterized antigenically, the incidence of infection can be determined by this method. Also, V antigen, as described by Lief and Henle,[7] might be used for strain-specific diagnosis by complement-fixation. Unless the strain is an antigenic newcomer like the Asian influenza virus, hemagglutination-inhibition antibody titer increments may not identify the subtype. Despite the initial difficulties the Asian virus presented, several methods of determining hemagglutination-inhibition antibody were found to be more or less satisfactory as long as the method of treatment of the serum was correlated with the strain of virus used. An inhibitor-insensitive early-passage virus and serum treated by heat alone were used by Hilleman,[8] while Jensen enhanced sensitivity to antibody as well as inhibitors by passages in the egg, ferret, and mouse (EFME line), and then treated serum with potassium periodate[9]; an antibody-sensitive strain that was not inhibited nonspecifically with sera treated with receptor-destroying enzyme of *V. cholerae* (RDE) was described by Choppin, Osterhout, and Tamm,[10] and monkey kidney culture propagated Asian virus and receptor-destroying enzyme treated sera was used in our laboratory.[9] Perhaps a far more important consideration than the particular technique employed is the need for each laboratory to evaluate the efficacy of its own procedures in terms of all observations and to express reliability as a function of a baseline. Thus, cases from which virus is isolated serve to evaluate serologic results, and those with antibody increments can be used for the isolation procedures. Different techniques can also be compared as shown in table 13. Either receptor-destroying enzyme or periodate was satisfactory for removal of nonspecific inhibitor of the tissue culture line, but only periodate was effective with the EFME line. The early passaged nonreactive fourth egg passage antigen failed to demonstrate antibody increments in half the cases if sera were treated with receptor-destroying enzyme, but was reliable with sera that had been treated only by heating at 56°C. for thirty minutes. Difficulties with these procedures occurred principally when the convalescent serum titers were very low and close to the threshold of sensitivity of these determinations. In such instances, though infrequent, the lines that were less reactive sometimes failed to reveal antibody in the convalescent serum. The differences in hemagglutination-inhibition antibody titers shown in table 13 dependent upon the virus line were also demonstrable by neutralization.

[7] LIEF, F. S., AND HENLE, W.: Bull WHO, 1959, *20*, 411.

[8] HILLEMAN, M. R., *et al.*: JAMA, 1958, *166*, 1134.

[9] JENSEN, K. E., AND HOGAN, R. B.: Public Health Rep, 1958, *73*, 140.

[10] CHOPPIN, P. W., *et al.*: Proc Soc Exp Biol Med, 1958, *98*, 513.

A baseline for comparison of antibody response following vaccination was also desirable because of the dissimilar procedures used by separate investigators. For this purpose the mean hemagglutination-inhibition antibody response following natural illness in a large group of persons was compared with the post-vaccination antibody titers determined by the same method.[11] Differences in titer could accordingly be used in comparing results of studies in other laboratories even when dissimilar virus strains or methods were employed. It was also noted that post-vaccination antibody titers determined with the EFME line were the same as those demonstrated with the tissue culture line, whereas titers with the latter were greater with post-influenzal serum.

Finally, the numerous procedures for isolation of the virus and serologic diagnosis of influenza should be used to advantage by providing the means for rapid application by different laboratories. To offset these variations in technique each laboratory should titer its own methods in terms of baseline observations. In this manner supplemental observations can be made. For special problems duplications of procedures will provide another type of information.

Dr. George K. Hirst (New York, New York): Since I have not worked on the problem of diagnosis in influenza for many years, I have no data to report and can make suggestions only to those who are actively trying to improve the methods. A very great effort has been put into serologic diagnosis, especially complement-fixation, and over the past fifteen years it has been proved over and over again that complement-fixation, properly done, gives a higher proportion of positive diagnoses than does hemagglutination-inhibition. Complement-fixation is very well suited to retrospective surveys of material collected during an epidemic and, with all of the refinements introduced, it has probably been developed to an efficiency close to the maximum possible for a serologic technique.

There is a very real need for a speedy diagnostic test which will be both efficient and fast, so that answers can be obtained within a few hours. I believe that such a method might be found in the combined use of tissue culture cells for growing the virus antigens and fluorescent antibody staining for specific detection of the antigens. There is nothing really new in this idea, and both tissue culture cells and fluorescent microscopy have been used for diagnostic tests; but I believe that they have not been employed under optimal conditions.

Influenza virus, even an established laboratory strain, often does not do well in established lines of tissue culture cells, at least in the sense that the lines produce little or no infectious virus. However, a large proportion of the cells from many lines will produce influenza *antigens* after exposure to influenza virus. Even if only a very small proportion of cells produced the antigen, it should be possible to devise a technique for scanning large numbers of cells for fluorescence. The finding of a few cells with antigen could be diagnostic. By limiting the requirement to antigen (not virus) production and examining large numbers of cells, it is conceivable that a specific test might be developed which is sensitive, specific, and quick in detecting influenza virus in human throat secretions.

Dr. Robert J. Huebner (Bethesda, Maryland): I am going to talk just a few minutes on influenza with respect to the rest of the problem. As most of you know, studies in our laboratory are continuous studies. They are mainly focused on children, and we discovered shortly after we started that we had to continue studies between the influenza outbreaks. Because we see the infants who are sick in the various populations, and cannot tell clinically what the viruses are, we end up studying other agents that we might not have intended to study.

In putting influenza in perspective we have to think about the total problem of respiratory disease, undifferentiated respiratory disease in particular. We have two or three general estimates of the total problem. One of them, and the most recent one, has been the National Health Survey, which in 1957–1958 estimated about 284,000 illnesses of respiratory nature which required medical

TABLE 13

Mean Hemagglutination-Inhibition Antibody Titers Determined with Tissue Culture and Egg Lines of Asian Virus*

	Virus				
	TC$_{2-4}$	E$_4$	E$_4$	E$_7$	EFME
Treatment of serum	RDE†	Δ56°C.	RDE	RDE	Periodate
Acute serum titer	<4	<4	<4	<4	<4
Convalescent serum titer	120	37	16	32	46

* Geometric mean titers determined from ten paired sera.

† Receptor-destroying enzyme.

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[11] Pelon, W., *et al.*: Proc Soc Exp Biol Med, 1958, *99*, 120.

care in this country alone. The Western Reserve studies—Dr. Dingle's group—show that memory is not a very good index of what happened in a previous year, and that this estimate is very low indeed. Perhaps a better estimate would be six to ten respiratory illnesses per person per year in this country.

This is by all odds man's largest morbidity problem, and certainly pneumonia is still something of a problem, since it causes death. The point I want to make is that influenza contributes only a certain portion of this. How much does it contribute?

I want to break down infections in children and eliminate chickenpox, measles, mumps, polio, and a great many other infections that have specific clinical signs. This still leaves a large number of undifferentiated illnesses. Drs. Bell and Davis and their associates concerned themselves with this problem over a period of some five years. Data are presented to show the weeks when the influenza virus was not isolated and the illnesses during weeks when influenza was isolated. These data point up the fact that the total amount of illness during the entire year could not be explained on the basis of the influenza alone. Actually, influenza accounted for only between 5 and 10 per cent of the illnesses. On the other hand, there was a rather uniform amount of total illness throughout the five years when influenza was not present. When it was present there was quite a bit of variation in the different age groups. In either case, particularly in the cases of non-influenzal infections, the problem appeared to be greater in children than in adults. Perhaps if one studied children less than seventeen years of age only, one might end up with almost as much illness as in all the rest of the population.

We have turned our studies primarily to one age group. This is one way of breaking down the total problem with respect to age. It seems rather apparent now that the infections that occur in children are going to be somewhat different in etiology than those that occur in adults. We have made an effort to explore the total problem over a period of almost two years in a study that is being done at Childrens' Hospital in cooperation with Dr. Parrott. Dr. Chanock's data show that influenza and other related viruses might account for perhaps 7 per cent of illness in one case, and 6 per cent in another, and that parainfluenza and adenoviruses accounted for considerably more. If these are added to other infections such as primary typical pneumonia, infections due to other respiratory and enteroviruses, a figure very close to 50 per cent is realized, which could be explained by available common agents during the respiratory seasons. We are monitoring the infections in this population, employing in our serologic tests some fifteen different respiratory disease viruses. These include five adenoviruses and three types of influenza virus. There is some monitoring for primary typical pneumonia, psittacosis, and Q fever. Just as soon as we can get our serologic tests working properly, we hope to add polio and all the rest of the viruses which we know occur and are contributing to the respiratory disease in these populations, particularly 2060 virus which has now been classified as ECHO 28.

We have an enormously large and complex problem with respect to respiratory disease and, when it is realized that from 50 to 100 viruses possibly contribute to this problem, I just want to make this point: We have yet to devote to these other non-influenzal and non-polio problems anything like the effort—the intensive effort—which is directed toward the influenza problem. These problems may be ten times as large or twenty times as large and certainly much more complex than influenza or polio. We must devote much more attention to these non-influenzal infections in order to understand or comprehend the total respiratory disease problem.

Dr. Irving Gordon (Los Angeles, California): Some five years ago I was at the New York State Laboratory in Albany. My colleagues and I were able to test the procedure suggested by Dr. Hirst with an analogous virus. We isolated five strains of measles virus by inoculating tissue cultures with respiratory secretions and recovered specific fluorescent cells in a few days. Later, typical cytopathic changes developed and the diagnosis was confirmed by the appearance of specific antibody in the serum.

I would also like to echo what Dr. Chanock said that, in such procedures, since the measles virus (as do the myxoviruses) have their simian counterparts, a large number of control cultures should be inoculated.

Dr. Karl M. Johnson (Bethesda, Maryland): I should like to amplify some of the comments offered by Dr. Chanock concerning the simian parainfluenza virus.

At present, monkey kidney cultures are widely employed by clinical investigators interested in viruses other than influenza. The current need for use of hyperimmune serum against the simian parainfluenza virus is therefore deplorable. Although we feel confident this virus is not associated with respiratory disease in this country, we have no information concerning its possible occurrence in other types of infectious diseases in man. As long as our cultures require this antiserum we will not find out. This difficulty as well as the implications for production of virus vaccines in monkey kidney cultures mentioned by Dr. Chanock

raise questions that deserve early, thorough study. Where and when do monkeys acquire their infections? What, if any, illnesses do they incur? How long is virus present in their kidneys? Are humans living in proximity to monkeys ever infected? To gain some insight, I think we shall have to go to places where monkey and man both are found.

Dr. Jonas E. Salk (Pittsburgh, Pennsylvania): Dr. Davenport, I am glad that you deferred the comments that I was going to make until now, because the data that were presented by Dr. Jensen and others certainly bear out and illustrate the point I was going to make. I have been thinking about the problem of the difference between the complement-fixation and hemagglutination tests with respect to specificity. It has been known for a long time that the complement-fixation test was less specific and, hence, better for diagnosis. The 1947 experience with the new influenza strain certainly emphasized this point. I thought the explanation of the problem resided in a possible difference in the mechanism of the two tests rather than merely in the difference in the nature of the antigens. Now it is quite clear that the complement-fixation test is based merely on the detection of the existence in the system of matching antigen and antibody. Hemagglutination, on the other hand, is based on the presence of antibody for the entire range of the hemagglutinating antigens. We can illustrate this in the following way.

If one has a serum that contains influenza A antibody to which is added a mixture of influenza A and B virus, the complement-fixation test will be positive. On the other hand, the hemagglutination-inhibition test will be negative because the B virus will agglutinate the erythrocytes in spite of the fact that the influenza A antibody will neutralize the influenza A antigen. Conversely, an anti-B serum and a mixture of A and B virus would be positive by complement-fixation but negative by hemagglutination-inhibition. On the other hand, a serum that contains influenza A and B antibody will be positive both with complement-fixation and hemagglutination-inhibition against a mixture of A and B viruses.

The point that I want to make here is that it is entirely understandable that the complement-fixation test would be better to show the existence of relationships between strains, whereas the hemagglutination-inhibition test would show the differences between strains. Consequently, the complement-fixation test would be clearly useful for diagnosis. However, the study of strains for inclusion in a vaccine, the hemagglutination test, and especially the test for neutralizing antibody, would be the most significant. Now, Dr. Jensen's data certainly illustrate this point very strikingly. He commented that the data referred to adults and not children; and in adults who were vaccinated there seemed to have been more complement-fixing antibody existing in the pre-vaccination sera than hemagglutination-inhibiting antibody. In post-vaccination sera the response was quick as if a booster effect had been induced by a common antigen. On the other hand, the hemagglutination-inhibition rise was both slower and much more specific.

Now, Dr. Liu's data and the point brought out by Dr. Lennette are all in keeping with this interpretation. It is also understandable, therefore, that the complement-fixation antibody would broaden with age; but that the hemagglutination-inhibition antibody would show the breaks that were shown by Drs. Francis and Davenport and also by Professor Mulder.

While I might say, in concluding, that a virus safari would be both advantageous and intriguing, it seems to me that the simple hypothesis of the human origin of these different strains may lead to the control of influenza somewhat more realistically than the search for the animal reservoir. But I think, Dr. Andrewes, it would be a good excuse for a trip to China, anyway.

Dr. Florence Lief: I would like to add to Dr. Salk's comments on the complement-fixation test and its relative specificity in comparison with the hemagglutination-inhibition test, particularly in regard to its usefulness in serodiagnosis. First of all, concerning soluble antigen and the complement-fixation test as performed by Dr. Jensen: he is perfectly right—one has to consider the age of the individual when interpreting the results. It is well known that soluble antigen is a weak antigen as shown by the fact that antibodies to it appear in only a small proportion of animals given *one* inoculation of live virus. Similarly, very young children, who are possibly suffering their first influenzal attack, do not respond uniformly by developing the antibody. Dr. Sohier of Lyon and we have found that, in children younger than four years of age, less than 50 per cent develop anti-S during convalescence. I should like to point out, too, that about the same proportion also develop strain-specific antibodies to one or more homotypic viruses in addition to that of the infecting strain. Therefore, in very young children soluble antigen cannot serve to advantage for serodiagnosis. However, all persons, regardless of age, show rises to the viral or V antigen of the infecting strain.

After the age of four, antibodies to soluble antigen rise, no doubt, as a recall response, the initial immunization having been an earlier influenzal infection. It is not surprising, then, that it commonly appears before anti-V to the infecting strain. Usually anti-S is discernible between the seventh and fourteenth days, while the strain-specific anti-V

to the infecting strain might take two to three weeks after onset to show a significant rise. Since Dr. Jensen uses infected allantoic fluid as antigen for complement-fixation, it can easily be seen why, in persons older than four years, he can arrive at a diagnosis earlier than when the hemagglutination-inhibition test is used, as influenza-infected allantoic fluid contains soluble as well as viral antigens.

It must be borne in mind, however, that allantoic fluid cannot be used for strain-specific diagnosis by complement-fixation of human sera. In such a system the S- anti-S response which is type specific obviously masks the strain-specific reaction. Therefore, the specificity of the hemagglutination-inhibition and strain-specific complement-fixation tests cannot be compared unless the proper reagents are employed. For human serodiagnosis this means that elementary bodies free of contaminating S must be used as antigen. Under such conditions it is easy to demonstrate that anti-V, like anti-hemagglutinins, rise later than anti-S. We feel strongly, however, that the complement-fixation test can be more sensitive than the hemagglutination-inhibition test in uncovering rises in strain-specific antibody since sera do not have to be processed before use. It is well established that treatment of sera with RDE, trypsin, periodate, or combinations thereof, may destroy low concentrations of serum antibody. It is perhaps for this reason that, in our hands, it has been possible to establish strain diagnosis by use of the complement-fixation test in better than 90 per cent of the sera from clinical cases of influenza submitted to us during the 1957–1958 epidemic.

Dr. H. F. Maassab (Ann Arbor, Michigan): I would just like to comment about another system that I have been using to isolate influenza virus. Miss Elva Minuse and I did a comparative study using embryonated eggs and chick kidney cultures for isolation of virus from 45 specimens.

Of the 44 specimens tested, 26 were positive in embryonated eggs and were identified as Asian influenza viruses. Thirty-four of the 44 specimens were positive in kidney cultures. Twenty-eight of these isolates were typed as Asian influenza viruses. Six of them were not influenza viruses, and they are not yet identified. Of the 6 unidentified viruses, 4 were positive by cytopathogenic effect (CPE) and hemadsorption, while two were positive only by CPE. The chick kidney culture system has proved to be as sensitive as embryonate eggs for routine diagnostic studies. The practical usefulness and efficacy for primary isolation of viral agents is apparent. In addition, the system offers economy and ease of operation.

Dr. Joseph A. Bell: I should like to elaborate a little bit on what Dr. Huebner discussed. First, Dr. Stuart Harris showed that we could not differentiate influenza of the various types clinically. Dr. Huebner extended this and stated that there are many other acute febrile respiratory diseases of two or more days' duration which likewise cannot be distinguished clinically. I think we have to look at the problem in perspective and emphasize that influenza is important because it hits all at once. In a matter of six, seven, eight, nine, or ten weeks, it goes through the community and disables a lot of people. Although the case fatality rate is rather low, this high concentration of a lot of cases gives sharp peaks in mortality, as shown by Dr. Collins.

If we stop to look at all the rest of these diseases which are not occurring characteristically in peaks, they also have a considerable mortality which is spread over the whole year. Just because they are not dramatically forced on our attention is no reason why they should be ignored. I think more effort should be made toward a study of these other diseases, but I do not wish to belittle the problem of influenza, which is close to my heart.

Dr. Scott B. Halstead (Washington, D.C.): I just want to make a very quick comment in defense of the hemagglutination-inhibition test in the diagnosis of Asian influenza. In 1957, Dr. Gresser and I (AMA Arch Intern Med, 1959, *103*, 590) did a pilot study at the 406th Medical General Laboratory in Japan in a group of 20 to 30 military personnel with serial bleedings at zero to three or four days, at twenty-one days, and then at six weeks, following infection. We then tested the sera simultaneously by the complement-fixation and the hemagglutination-inhibition tests using a strain of influenza which was relatively antibody sensitive and non-specific inhibitor insensitive. We found that the geometric mean titers of hemagglutination-inhibition antibody rose rather significantly during the interval from twenty-one days to six weeks. A little more than half of the serum specimens were hemagglutination-inhibition negative at twenty-one days, but positive at six weeks. Many of these cases were documented by recovery of influenza virus from throat washings. The point I would like to make is that the hemagglutination-inhibition test may still be useful in Asian influenza if late convalescent-phase sera are obtained.

CRITIQUE OF DIAGNOSTIC SYSTEMS

FRED M. DAVENPORT, *Chairman*

INFLUENZA: DIAGNOSTIC PROCEDURES AND LABORATORY ORGANIZATION, 1957[1]

MORRIS SCHAEFFER[2]

When the current influenza A virus variant emerged in the Orient in 1957 and began the rapid global dissemination of its progeny, about twenty-five years had elapsed since the first isolation of influenza virus had been demonstrated in the laboratories of Andrewes in England and Francis in this country. In the interim, these men continued their vigorous investigation, and with their colleagues and students were shaping the tools and techniques which are responsible for our present knowledge of influenza.

Simplified methods of working with viruses attracted additional men of talent. Notable among them was Hirst who, with his discovery of the hemagglutination phenomenon, presented us with an extraordinary device. For here was a method capable not only of providing certain basic information about the virus and its interaction with host cells, but also one with which virus variants could be recognized more precisely than was hitherto possible. Armed with these resources, imaginative workers rapidly developed efficient diagnostic procedures with which the influenza viruses became the easiest and best studied in the laboratory and in the field.

At the outset of the pandemic in 1957, therefore, there was a small but seasoned group of workers prepared to apply their sharpened tools and improved concepts to the tasks at hand. Thus, it is not surprising that with relatively few tests they promptly recognized that influenza virus isolates from Hong Kong, Singapore, and Japan belonged to a new set of type A variants composed of a significantly different antigenic structure.

Confirmation of the prevalence of this new strain soon came from other laboratories as cases rapidly mounted into the millions and the virus spread in every direction. Experienced and interested workers in public health, military, and university laboratories went into immediate action, testing and exchanging material and communicating their results freely and rapidly.

At this point, it was considered necessary to mobilize all available laboratory facilities. To accomplish this there were fortunately several laboratory organizations specifically established for the anticipated eventuality of a pandemic. The Commission on Influenza of the Armed Forces Epidemiological Board had established listening posts in strategic locations and a Strain Study Center in New York at the end of World War II.

In 1947, through the foresight of Dr. C. H. Andrewes, an International Influenza Center sponsored by the World Health Organization was set up under his direction at the National Institute for Medical Research in London. Its objective was to improve specific diagnosis and reporting of influenza by the formation of a network of laboratories which were capable of recognizing virus variants. Such virus isolates would then be submitted to the Center for strain analysis and further study.

The next year, following Dr. Andrewes' invitation to the Surgeon General of the United States Public Health Service to participate in a cooperative program, a World Health Organization Influenza Study Program was organized in the United States, which operated under an interservice advisory committee consisting of representatives of the Army, Navy, Air Force, Public Health Service, and Bureau of Animal Industry of the Department of Agriculture.

The laboratory network consisted of the existing Strain Study Center of the Commission on Influenza under Dr. T. M. Magill and about fifty civilian and military laboratories selected with reference to geographic coverage and their facilities and interest in investigating influenza.

In 1955, Dr. Magill asked to be relieved of his responsibility for the Strain Study Center. This work was undertaken for the Commission on Influenza by Dr. Fred M. Davenport at Ann Ar-

[1] Communicable Disease Center, Virus and Rickettsia Section, and International Influenza Center for the Americas.

[2] Present address: Bureau of Laboratories, Department of Health, New York, New York.

bor, Michigan, while the WHO Strain Study Center activity was moved to the Communicable Disease Center, Virus and Rickettsia Section, in Montgomery, Alabama, where Dr. Keith E. Jensen was brought in to assist in developing it. In 1958 the Montgomery laboratory was designated as the International Influenza Center for the Americas and the interservice advisory committee was dissolved.

The International Influenza Center for the Americas kept in close contact with the WHO laboratory in London, the Commission on Influenza at Ann Arbor, the military laboratories, and other active laboratories in health departments, universities, and pharmaceutical firms. At the same time it extended the functions of its program so that it:

(*1*) Expanded the network of collaborating laboratories in this hemisphere to 135, including forty-five in state health departments, thirty-nine in universities, eleven in research institutes, eight in hospitals, three in military laboratories, six in municipal laboratories, and three in pharmaceutical concerns. Twenty of these laboratories were outside the United States; three of these were located in Canada, four in Central America, and thirteen in South America.

(*2*) Performed antigenic analysis of newly isolated strains of influenza virus submitted by collaborating laboratories.

(*3*) Produced and distributed standardized influenza diagnostic reagents. By June, 1957, 15 ml. of each virus antigen and 5 ml. of serum were distributed to more than 100 laboratories. By October, 1957, 30 liters of antigen and 10 liters of serum had been distributed to more than 300 laboratories.

(*4*) Disseminated current information concerning influenza isolation, typing, and serologic procedures to collaborating laboratories.

(*5*) Served as a center for accumulation of current information on the status of influenza in cooperation with the Influenza Information Center at the National Office of Vital Statistics and the Surveillance Section, Epidemiology Branch, Communicable Disease Center.

(*6*) Offered diagnostic services when needed, and conducted a survey for the evaluation of diagnostic procedures as performed in various official health agencies.

(*7*) Evaluated current laboratory techniques for influenza diagnosis.

(*8*) Evaluated vaccines for use prior to, and during, the pandemic of Asian influenza in North America.

(*9*) Maintained an influenza virus type collection for distribution to interested organizations.

(*10*) Participated in a survey of influenza in swine and horses.

(*11*) Carried out serologic surveys in conjunction with special community studies.

(*12*) Participated in a study of the possible effects of Asian influenza in pregnancy.

(*13*) Participated in training programs by holding influenza workshops for laboratory personnel, offering formal courses for select groups, and providing specialized training sessions on an individual basis.

The diagnostic procedures employed by most of the laboratories, with the inevitable minor modifications of their own, have been amply discussed by the previous speakers and are well documented in the literature. In developing the program for the laboratory network, attempts were made to follow standard or generally acceptable techniques whenever possible. Frequent memoranda were issued to collaborating laboratories to point out pitfalls, particular sources of trouble, or changes in technique found necessary for satisfactory results.

There were, of course, the unavoidable squabbles among the experts regarding the most effective means of removal of nonspecific inhibitors from serum; which strains, passed through which hosts, provided the best antigens; the hemagglutination-inhibition versus complement-fixation test for serologic diagnosis; how to designate new isolates; and other imponderables. Most of these points were resolved, at least temporarily, as tempers cooled and the questions were re-examined objectively.

These experiences (echoes of which were heard again today) merely serve to emphasize the well-recognized fact that current methods, while unquestionably useful, are not without their limitations. So much more information is needed to fill in the many gaps in our incomplete knowledge of the influenza viruses and their behavior before we can design suitable studies for the development of more rapid and reliable diagnostic procedures.

Even the seemingly simple matter of the collection, preservation, and shipment of specimens can at times become a complicated and vexing problem, especially for material in prolonged transit. Dry ice, special containers, regular and rapid transportation schedules, and prompt delivery are all very fine and helpful

when you can get them. In routine practice, however, something often goes wrong somewhere along the line and the procedure is usually "fouled up."

In this regard it may be of interest to mention an unusual experience encountered in our laboratory with two shipments of throat washings, one from Chile, the other from Hong Kong. Although they arrived on different occasions, warm, putrid, and foul, two or three weeks after they were expected, several of the specimens yielded A2 influenza virus by ordinary isolation procedures. This would indicate that influenza virus may be somewhat more hardy than it has been believed to be. If it was capable of survival under such severely adverse circumstances, should we not be capable of devising a relatively simple and effective method of preservation? Surely, there must be something less expensive, cumbersome, and impractical than dry ice! But, to date, there has been no report of any attempt at a systematic search for a practical physical or chemical method of preserving virus in tissue or body fluids at room temperature or higher. Such investigations appear prosaic and unexciting, yielding always to the more appealing fundamental problems.

From an objective appraisal of the value of the organized network of laboratories during the pandemic of 1957–1958, the following prominent facts emerge:

(*1*) The essential work was performed by a small group of experienced and reliable workers, most of whom had been engaged or interested in influenza studies for many years. Only a small number of additional dependable laboratories could be recruited.

The basic laboratory results were derived chiefly by application of the "true and tried" techniques, but even these presented some difficulties, indicating areas requiring further study.

(*2*) Of the large number of persons hastily gathered and trained for the expanded laboratory network, only a few were capable of making (and still fewer actually made) significant contributions to the virology or epidemiology of influenza during this period. However, the program did attract a few competent persons who otherwise may not have been stimulated to enter the field of virology. Moreover, there was a sense of security in the knowledge that wide geographic coverage and a surplus of alerted laboratories were available.

(*3*) A crash program is of limited value when a pandemic strikes. It can accomplish only as much as the utilization of existing knowledge by prepared personnel will permit. Preparation for the next pandemic should include sustained activity in applied as well as basic research, integrated with a broad training program during the ensuing interval.

DIAGNOSTIC LABORATORY ORGANIZATION: FUTURE[1]

C. H. ANDREWES

Anyone interested in influenza must have at the back of his mind a fear of another "1918." A main object of diagnostic laboratories is to spot as quickly as possible a new strain of influenza virus which might be the seed of a lethal pandemic. Are we satisfied with our present spotting system? To serve the ideas we have in mind, such a system must certainly be world wide. Today, the networks of laboratories in the United States, in Britain, and in a few other countries are working well and swiftly. That, however, is not enough. In 1957 the Asian virus outwitted us by turning up in China, in the one part of the world to which WHO's laboratory-network did not extend. Laboratory workers in China undoubtedly identified the A2 virus as a novel strain before anyone else did so, but this knowledge did not quickly reach the rest of the world. Only when the new virus spilled over from China to Singapore and Hong Kong did we discover what was happening.

In Britain, foot and mouth disease is not endemic, but reaches us in devious ways either from the European continent or in materials imported from South America. Our best means of keeping it out of Britain is judged to be by improving methods of vaccination. Vaccines so developed would not be used in our own cattle, for then the disease would become endemic with us, also; but to help our near neighbors and others further afield to control *their* foot and mouth disease. We thus hope to keep the virus at arms' length. We cannot do just this with influenza, but I believe that we can do more good to everybody, including ourselves, not by perfecting our already good arrangements at home, but by working towards an efficient world-wide system. The World Influenza Centre at Mill Hill tries to keep in touch with Europe, Asia, Africa, and Australia, leaving North and South America to be looked after by the Communicable Diseases Center at Atlanta, Georgia.

In Europe we do fairly well with twenty-nine laboratories; but in Asia, apart from the Soviet Union, there are only eleven, in Africa seven, and in Australasia six. That makes them rather "thin on the ground." I can well imagine that there are many vast areas in the world, nominally under the aegis of WHO, where an epidemic due to a new influenza virus could rage for several weeks without our getting to know about it. In many tropical countries, for instance, they don't bother very much about influenza in most years, though they are heavily hit by big pandemics, as in 1918 and 1957. In other years, curiously enough, they may have outbreaks at almost any season and these may be quite important for the study of epidemiology; yet our centers in the tropics are very few.

Even in countries with reasonably good laboratory facilities it is believed that there is scope for prompter diagnostic work. During the recent influenza in Europe, I have had very many 'phone calls from reporters and have blushed again and again as I have replied that so far our only source of information has been the daily press.

How much do we want our peripheral laboratories to be able to tell us? If they report, as they have done this year, that current viruses are just like the recently prevalent A2—or at any rate very similar—a delay of a week or two does little or no harm. We do need, however, to persuade all influenza workers to send viruses (dried, frozen, or glycerinated) to main centers as quickly as possible if there is the least suspicion that they are novel. The glory of being the first discoverer of a novel strain in Ruritania and describer of its detailed properties is not so very great, and it is nothing to the glory of having distributed it as quickly as possible to where it is badly needed.

We next consider the laboratory on the fringe of things which may happen upon an apparently new strain. It may chance, as in 1957, that the strain behaves oddly in its susceptibility to sundry inhibitors, so that the unwary are led astray. Or if they don't know enough about nonspecific inhibitors or about P-Q variation, they may mistake an old strain for something new. Is the deceptively easy hemagglutination-inhibition test best discarded for something with fewer pitfalls? The complement-fixation test of Lief and Henle (1959) against V antigens would seem to have

[1] National Institute of Research, Mill Hill, England.

great advantages for specific identification of virus isolates, though, of course, when used in the study of human serum it gives less specific results. Preparation of the necessary reagents is, however, rather tedious and difficult and the widespread use of the complement-fixation test instead of the hemagglutination-inhibition test may depend on the possibility of wide distribution of reagents made commercially. We must be on the look-out, too, for other tests to make diagnosis simple and certain, even at the ends of the earth. Even if we decide to stick to conventional hemagglutination-inhibition and complement-fixation tests, there is great importance in making reliable reagents available to people who cannot readily prepare them or standardize them themselves.

I will now venture outside the strict confines of our subject, Asian influenza. It is wasteful to set up an elaborate organization to deal only with influenza. Fortunately, WHO is taking a serious interest in extending the scope of its influenza network to include other respiratory viral infections. We have no reason to suppose that, just because its activities are so obvious, influenza A is the only virus which takes the whole world as its parish. Maybe it *is* unusual in this respect: we don't know until we inquire. There is no doubt that some of the ECHO viruses, particularly ECHO 9, become troublesome in diverse areas at about the same time. So too, adenovirus type 7 began to cause more trouble than types 3 and 4 about the same time on both sides of the Atlantic.

New respiratory viruses are being described almost every week. These are certainly going to turn up in different parts of the world and be given different names by their discoverers: this happened with Sendai virus alias hemagglutinating virus of Japan alias newborn pneumonitis virus alias influenza D—now, I hope, finally named "parainfluenza 1" by international agreement. A mechanism is badly needed for ensuring comparison of "new" viruses and the avoidance of confusion in naming. I am talking now of respiratory viruses, but there is need, too, for development of the existing nuclei for sorting out enteroviruses and arborviruses.

As I see it, the structure needed, in the case of the respiratory viruses anyway, can be conceived of as in three tiers. Tier one, in the front line, consists of existing virus diagnostic laboratories of all kinds and of varying degrees of efficiency. If these isolate a virus which they cannot name, they can refer it to second-tier laboratories, which may well be incorporated with some of the better-equipped, more experienced, WHO influenza laboratories. These will have to be supplied, by WHO or from some other source, with an extensive set of diagnostic reagents, mainly sera, prepared against a wide range of viruses. If they, in turn, cannot identify the agent in question, recourse must be had to third-tier laboratories. There would probably be only three or four of these, one perhaps serving as the authority on adenoviruses, another on myxoviruses, and so on. Top virologists don't want to spend all their time doing diagnostic work. The staffs of the *tier 3* laboratories would accordingly be mainly research workers, appealed to by *tier 2* laboratories only infrequently. They would, however, have to be responsible for the authenticity of the reagents distributed to *tier 2* laboratories and made, one hopes, commercially. Behind them would have to be an international committee, to resolve difficulties. This could usually be done by correspondence; meetings would be quite rare. The *tier 3* laboratories would work out an arrangement between themselves whereby new viruses were appropriately named. They would doubtless keep in close touch with the International Nomenclature Committee for Bacteria and Viruses.

We have in the respiratory infections a problem of major importance for industrial efficiency and human happiness. Knowledge in the field is advancing so fast that we must take care not to be confused, entangled, and overwhelmed. The influenza laboratory networks, national and international, have been planned with care and are beginning to work better and better. Upon this system can be grafted something which can codify our knowledge of other respiratory viruses, as it accumulates, and so lead toward the goal of their ultimate control. Influenza diagnostic laboratories need not confine their attention to influenza, nor need they concentrate only on diagnosis but should help also in the design and evaluation of attempts at preventing respiratory disease.

General Discussion

Dr. Hideo Fukumi: There are 406 prefectures in Japan each of which has its own health laboratory. Generally speaking, the prefectural health laboratory shows fairly good effectiveness in bacteriologic examinations, especially in examina-

tions of Enterobacteriaceae, but its virologic facilities are quite poor. However, every prefectural laboratory must cooperate in influenza examinations for the National Influenza Center that is located in the National Institute of Health of Japan. In this respect, the prefectural laboratory is principally required to be able to carry out hemagglutination-inhibition reaction and complement-fixation reaction, if possible; but virus isolation is carried out in the National Institute of Health, which is equipped to receive pathologic materials from every part of the country. Of course, several of the prefectural laboratories are able to do virus isolation work, and in such cases they are requested to send isolated viruses to the National Center for typing.

However, in some of the prefectural laboratories the results of hemagglutination-inhibition titrations are not always reliable and, according to my experience, one of the reasons for such unreliability occasionally seems to be unsatisfactory or inadequate treatment of nonspecific inhibitors. In this respect, it would be most convenient if we could obtain a strain not inhibited by nonspecific inhibitors present in human serum. For this purpose, we originally collected a number of strains of A2 influenza virus which showed the characteristic of noninhibition by horse serum and of good inhibition by specific A2 antibody. About twenty such strains were chosen, but most of them were found unsatisfactory when used for titration of human serum because they were more or less inhibited by nonspecific inhibitors, though not inhibited by horse serum. However, there was a strain (Kagoshima 9) which was almost satisfactory for the purpose mentioned above. Approximately the same values of hemagglutination-inhibition titer were obtained for human serum, when tested before and after treatment with receptor-destroying enzyme (RDE), and this strain was found not to be inhibited at all by nonspecific inhibitors in human serum. A comparison of the values of hemagglutination-inhibition titers of human serum estimated by Kagoshima 9 strain before and after treatment with receptor-destroying enzyme and by Adachi strain, the strain most commonly used in laboratories in Japan for A2 influenza virus, reveals that the Kagoshima 9 strain is fairly satisfactory for the purpose mentioned above.

In addition, we were able to obtain a mutant line of Adachi strain, which is not inhibited by nonspecific inhibitors in human serum, by growing the virus in embryonated eggs in the presence of human serum containing no specific A2 antibody, as mentioned by Dr. Robinson this morning. We are now examining it to learn if it is also of use for our purposes.

Dr. E. H. Lennette: Drs. Schaeffer and Andrewes have so comprehensively covered their subjects that there appears to be little more to be said. However, Dr. Schaeffer has made several interesting observations upon which I should like to enlarge briefly, and Dr. Andrewes has mentioned organization of a surveillance network, upon whose structure and functions I should like to comment.

Despite the dismay and hand-wringing attitude expressed in certain quarters, the appearance in 1957 of a new strain of influenza virus (one markedly different antigenically from its predecessors) was not an unexpected event or an unforeseen contingency for which no preparations had been made or to which no thought had been devoted. There was in the United States, for example, the important but unpublicized research conducted by investigators associated with the Commission on Influenza of the Armed Forces Epidemiological Board; comprehensive studies on influenza have been carried out under the aegis of the Commission continuously from early in World War II up to the present time. There was, in addition, an international network of laboratories and surveillance centers organized by the World Health Organization and charged with the specific mission to detect the appearance of influenza; to isolate and analyze the antigenic composition of new strains so that, should such antigenically new strains appear, they might promptly be utilized for the preparation of highly strain-specific vaccines.

I find Dr. Schaeffer's experience with a "crash program" rather illuminating. He points out that a large number of persons were hastily gathered and trained in order to increase greatly the number of laboratories in the influenza surveillance network in the United States; of this large number of additional laboratories, "only a few were capable of making (and still fewer actually made) significant contributions to the virology or epidemiology of influenza during this period." Dr. Schaeffer found such a program to be of practical value only when a pandemic struck, and even then it had severe limitations. Perhaps it might be better to reorganize such a program into one which will provide for sustained, rather than periodic, support of a laboratory, and permit it to engage in a continuous effort in the applied as well as basic research fields. However, it is my firm conviction that, as Dr. Andrewes indicated, we should give thought to establishing not additional laboratories concerned solely with influenza virus, but laboratories with the necessary background and interest to carry out both applied and fundamental research on the whole gamut of viral respiratory diseases.

To carry out such a program, viz., one concerned with a variety of respiratory disease agents possessed of markedly different properties and characteristics, it becomes necessary to consider the

type of structure that Dr. Andrewes has mentioned. Indeed, similar or identical problems exist in the world-wide study of the enteroviruses and the arthropod-borne viruses. In the case of the latter group at least, an informal, loosely organized group of investigators in the United States has already done much planning toward the eventual establishment of local and regional laboratories and international reference centers along lines similar to those mentioned before this meeting. The local laboratories, or the *tier 1* laboratories of Dr. Andrewes, would be in the forefront; this group would be comprised of the existing virus diagnostic laboratories "of all kinds and of varying degrees of efficiency." These would be backed up by the second-tier and third-tier laboratories mentioned by Dr. Andrewes. On paper, at least, the functions and duties of all three tiers are simple to delineate and enumerate, but in practice numerous problems arise. Time permits me to mention only one or two.

Thus, who is to prepare the requisite diagnostic antigens, antisera, and reagents, and who is to bear the cost of their manufacture? To whom should they be distributed, and how should they be utilized to avoid wastage—a mundane problem to be sure, but an important economic one. How far should a *tier-1* laboratory proceed in its identification attempts before passing the agent on to a *tier-2* laboratory; how far should the *tier-2* laboratories proceed in their identifications? I think that we shall eventually arrive at a world-wide network of surveillance laboratories for viral respiratory disease, similar to those being contemplated at the present time for the arborviruses; but many problems, administrative as well as technical, will have to be resolved before such a network becomes a practicality. These problems will demand, aside from time, much devoted effort and thought.

Dr. F. P. Nagler, (Ottawa, Canada): The Canadian system for the laboratory diagnosis of influenza epidemics is based on the cooperation of reporting and specimen-collecting agencies in all provinces, with the central information and strain-typing centers at the Department of National Health and Welfare's Virus Laboratories. These agencies are predominantly represented by the provincial laboratories of health, the Federal Department of Veteran Affairs, and the Immigration Medical and Indian Health Services of the Federal Department of Health and Welfare. They have been supplied with equipment and shipping containers for the collection of representative specimens and go into action as soon as an outbreak has been reported by the Medical Officer of Health. Specimens are shipped in frozen condition by air to Ottawa where attempts at virus isolation and identification are commenced immediately. In several cases provincial laboratories are well equipped and carry out virus isolation procedures simultaneously. Samples of virus isolates are forwarded to the WHO centers in London, England, and Atlanta, Georgia, as soon as they have been identified as belonging to the influenza group.

SPECIFIC PROPHYLAXIS

COLIN M. MACLEOD, *Chairman*

INACTIVATED INFLUENZA VIRUS VACCINES[1, 2]

Past, Present, and Future

FRED M. DAVENPORT

The value of vaccination against influenza was never before as clearly acknowledged as it was by the emphasis placed on vaccine production in the efforts made during 1957–1958 for defense against Asian influenza. This symposium provides an opportune occasion for appraising the meaning of that effort and—more importantly—for formulating answers to the question: "Where do we go from here?"

It is not my intent to review the historic development of influenza virus vaccines, since that subject has been extensively covered elsewhere (1, 2). Rather, it seems more pertinent today to attempt to epitomize past accomplishments in a manner that sets in relief certain of the crucial problems encountered, of the concepts developed about them, and of the steps taken to find solutions.

For convenience, I have chosen as a text the accumulated results of the field trials carried out since 1942 by the Commission on Influenza. The findings have been in large part confirmed by less extensive studies conducted independently in this and in other countries (figure 1). In the data presented, the protective effect found in each study is summarized as the ratio of the percentage of attack rate in controls to that of vaccinated subjects.

The results of the first trial of 1943 against influenza A exceeded the expectations of the investigators involved. An average protection ratio of 3.6 was found. In 1945, as will be illustrated later, the efficacy of vaccination against influenza B was proved with equal firmness. Yet despite these encouraging results, it was realized that certain questions remained unanswered. Paramount among them was: "Are strain differences measured serologically of importance for protection of man by vaccination?"

The results of the first two field trials did not provide an answer, since the vaccine used in 1943 contained a recent type A isolate not very different antigenically from the strain encountered in the test, while the Lee vaccine given in 1945 was quite different serologically from the type B virus involved in the epidemic of that year. Obviously, further trials were indicated.

A decisive answer came in 1947 when influenza A1 unexpectedly appeared in the Northern Hemisphere. Vaccination with a preparation deficient in A1 antigens proved to be totally ineffective. This result was of prime importance in focusing attention on the significance of antigenic variation in relation to immunity in man. Further, it intensified on a global basis interest and efforts for rapid identification and antigenic analysis of each year's prevailing virus.

The immediate problem of vaccine failure against influenza A1 was resolved by incorporating representative members of that family of strains in all subsequent vaccines. Between 1950 and the summer of 1957 an important degree of protection against influenza A1 was uniformly produced by vaccination even though minor antigenic differences in the prevailing strains continued to occur throughout the period. The protection ratios obtained with vaccines of different composition varied between 3.1 and 8.1.

The lessons learned in 1947, and subsequently, provided a firm basis for recommending vaccination with Asian strains when the 1957 pandemic threatened. Yet, obviously, the validity of the recommendation could only be established by testing, and for this purpose field tests were conducted by the Commission on Influenza during the summer of that year. The results were decisive. Protective ratios varying between 1.7 and 4.4 were found, depending on the potency of the product given.

[1] From the Department of Epidemiology and the Virus Laboratory, School of Public Health, University of Michigan, Ann Arbor, Michigan.

[2] Portions of the investigation were conducted under the auspices of the Commission on Influenza, Armed Forces Epidemiological Board, and were supported by the Office of the Surgeon General, United States Army, Washington, D. C.

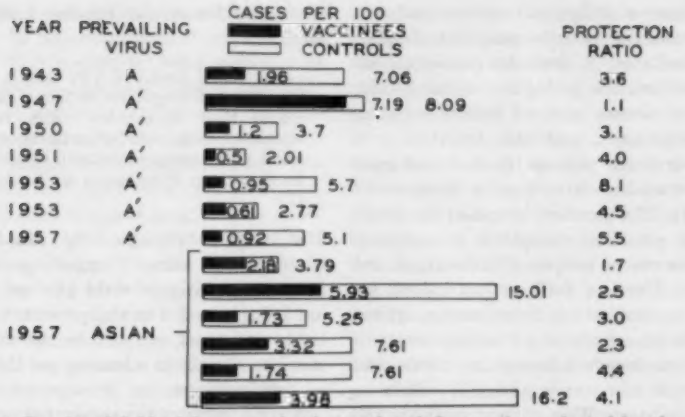

FIG. 1. Results of field trials on vaccination against influenza A, conducted by the Commission on Influenza, Armed Forces Epidemiological Board.

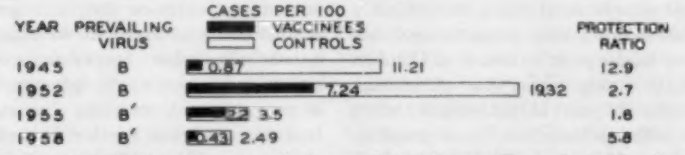

FIG. 2. Results of field trials on vaccination against influenza B, conducted by the Commission on Influenza, Armed Forces Epidemiological Board.

There have been relatively few opportunities to conduct tests of influenza B vaccines, yet the results obtained to date follow a similar pattern (figure 2). In 1945, using vaccines containing the Lee strain of 1940, a protection ratio of 12.9 was found. In 1952, a ratio of 2.7 was observed in a small trial of aqueous Lee vaccine carried out at a children's institution in Michigan. In the larger trial of 1955 among the military, using the more potent mineral oil adjuvant Lee vaccine, a relatively poor amount of protection was obtained. The ratio was 1.6. With low-dose aqueous Lee vaccine no significant effect was found that year. This experiment was part of a purposeful plan in which the antigenicity of strains of influenza B isolated in 1940, 1946, 1952, and 1954 were being tested in man (3). The serologic and the epidemiologic data gained provided a factual basis for up-dating vaccines by the addition of a 1954 type B isolate. The benefit conferred thereby was demonstrated in a field trial conducted at the Great Lakes Naval Training Center in the fall of 1958. Aqueous vaccine containing the 1954 Great Lakes B and the Lee strains yielded a protection ratio of 5.8[8] By analogy to influenza A, the strains prevalent in 1954 and since may be designated as B1 isolates, although the degree and the rate of antigenic change among type B viruses appear to be less than among type A.

Clearly, the data illustrated, and those reported from many other studies carried out in many other lands, unequivocally establish the fact that vaccination against influenza A and B is an effective procedure when vaccines of proper constitution and potency are employed.

So much for the past. Now, what of the present? While the success obtained with influenza virus vaccines is striking, there remain problems that are as yet not fully resolved. Foremost is the problem of how best to provide at all times by vaccination effective protection against viruses that have the unique characteristic of seldom appearing twice wearing precisely the same antigenic overcoat.

There have been two views on the nature and

[8] The writer is indebted to Captain Lloyd F. Miller, Commanding Officer, Naval Auxiliary Medical Research Unit 4, for permission to use these data prior to publication.

the significance of antigenic variation and two different approaches toward coping with the phenomenon. One view is that the protection induced by vaccination is highly strain-specific, that influenza viruses have an infinite capacity to vary antigenically, and that variation is a progressive or linear process in which old antigens are lost while new ones arise *de novo* by mutation (4). The practice proposed by advocates of this school of thought is to vaccinate with the most recent isolates of influenza A and B obtainable. This is a dark outlook indeed, for it implies that the future of vaccination against influenza should consist of an endless series of crash programs designed to capture, bottle, and distribute each new minor antigenic villain as he mounts the stage. With current methods and technology, that approach is impractical. Despite the unprecedented effort made in 1957 to provide adequate quantities of Asian vaccine, only about 15 million doses were prepared and distributed prior to the peak incidence of October. At that time the supply fell far short of demand. It is obvious that, in years of less intensive effort and in years with less lead-time, the accomplishment will fall considerably below that standard.

Fortunately, there is an opposite view which leads to a more hopeful outlook for the control of influenza by vaccination. It has the additional advantage of being more consistent with current knowledge about the nature and extent of antigenic variation and about the relation of antibody to immunity in man.

First, what of the possibility that new antigenic variants will appear endlessly to plague us? It seems unlikely. Recent work on the antigenic composition of influenza viruses has led to the demonstration that with the passage of time old antigens are not commonly dropped nor do new ones, completely unrelated serologically to their predecessors, commonly arise. Rather than an infinite series of mutations, the mechanism of antigenic variation seems to consist of a shifting in dominance of a few of the antigens that apparently the majority of strains of a type contain in lesser amounts. The number of antigens, though large, seems finite. The results of the elegant antigenic analysis of type A strains reported by Jensen and Francis (5) and by Jensen and Peterson (6) provide a firm basis for rejecting the unidirectional mutation hypothesis of antigenic variation.

In commenting on the antigenic analysis described by Jensen and Francis, Andrewes wrote, in 1957:

> The wheel they feel may turn a full circle, so that old half-forgotten antigens may some day regain their importance. Only time can tell whether the number of antigens is limited and whether the progression will be along a straight line—or on an Einsteinian or "Franciscian" curve (7).

The evidence presented by Mulder (8) and confirmed by others, suggesting that at least some of the antigens of the 1890 pandemic strain are being recycled in the present Asian isolates, lends additional support to the view that the number of antigens is limited and that resurgence of "old" antigens can be expected.

Second, what of the thesis that vaccines ought always to contain the most recently prevalent strains? The results of the field trials provide unequivocal evidence that, as a general rule, it is unnecessary to vaccinate with the most recent isolates obtainable. For example, in 1952 significant protection was found against influenza B after vaccination with the Lee strain isolated twelve years earlier. Further, in the Commission's studies on influenza A1, vaccines were repeatedly used successfully that contained A1 virus isolated several years before the challenge. With respect to vaccines containing A1 viruses, the longest interval of which I am aware was reported from the 1956 field trials of the British Committee on Influenza (9). A preparation containing a 1947 isolate was found just as effective as one containing a 1955 strain. Clearly, in practice, important protection has been repeatedly demonstrated by vaccination with strains of considerable vintage.

Finally, what of the thesis that immunity after vaccination is highly strain-specific? The results of studies carried out in our laboratory have amply demonstrated that vaccination of man with either A, A1, B, or B1 monovalent vaccines in general yields high levels of antibody against all other members of the corresponding family or set of strains (10, 11). The serologic and the field trial data indicate that the response of man to vaccination is at least "family-specific" not "strain-specific."

Yet, immunity to influenza cannot be explained solely by the presence of "family-specific" antibody. Correlations made in 1953 on the development with age and repeated exposures of a composite antibody and a composite immunity against influenza, clearly point to the fact that

protection can be conferred by antibody heterologous to the major antigens of the epidemic strain (12). With respect to Asian influenza in 1957, the relative resistance of persons past their childhood could largely be attributed to the presence of antibody oriented to other families of influenza A strains, since Asian antibody was generally absent until the closing decades of life; yet the attack rate declined progressively after the age of twenty. Once again, the explanation that the relative immunity of the older cohorts of the population may be in large part attributed to the acquisition with age and repeated infection of a progressively broader composite antibody, rather than by the presence of strain- or even "family-specific" antibody, was substantiated.

From these observations, the logical goal for vaccination is to induce, by giving a composite antigen, a composite antibody and a composite immunity which imitates or surpasses that of the older portions of the population whose antigenic experience has been the richest. Translated into current practice, this means compounding a vaccine with strains antigenically representative of each of the four families of influenza A and of the two families of influenza B viruses. Additions to the coverage should be needed only occasionally when, as in 1946, 1955, and 1957, a new family of either type emerges. Such a vaccine formula has the attractive advantage of relative stability.

Now, what of the future? "Where do we go from here?" One crucial problem repeatedly experienced is a shortage of vaccine at a time when the need is greatest. This is not a simple problem, but at least two potential partial solutions warrant consideration. Grist in our laboratory in 1957 (13) and, more recently and more extensively, Kilbourne at Cornell (14) have demonstrated that the yield of recent isolates that are poorly adapted to eggs may be considerably enhanced by appropriate recombination of the new strain with an old one well-adapted to eggs. Such techniques may prove useful when there is the rare need for rapid production of vaccine for defense against strains of a new family of virus. In the case where change in the vaccine formula is not indicated, because the threatening strain is an "auld acquayntace," there is an alternative to crash programs of uncertain reliability: the deliberate creation now of "stockpiles" of vaccine in anticipation of future needs. It would be tragic indeed to have to suffer a severe visitation of influenza when the lead-time was too short to permit production of adequate amounts of a vaccine already known to be effective. Moreover, I would like to suggest that, in an emergency, use of "old formula" vaccine would probably yield better results than no vaccine, in the hypothetical case wherein an important new strain could be captured and tamed in time. The thesis of heterologous immunity engendered by composite antibody is obviously the basis for this suggestion. The report by Gundelfinger and associates that in 1957 a vaccine containing merely a swine and an A1 strain yielded significant protection against Asian influenza in a field trial is a practical demonstration of the principle (15). The size and the composition of the "stockpile" is a calculated risk, but the probability seems high that the expenditure would not be wasted.

With greater surety one can predict a marked improvement in the purity of the vaccines to be used in the future. The desirability of employing only the essential antigens in a killed vaccine is not a new idea (16). While in Tubingen, Germany, Drs. Rott, Schafer, and myself demonstrated that highly purified preparations of hemagglutinin could be obtained after ether treatment of swine, A, A1, and Asian viruses (17). These were proved in vaccination studies to be potent stimulators of hemagglutinating-inhibiting antibodies. More recent experiments carried out at Ann Arbor have been oriented towards the development of a methodology readily adaptable to commercial production. The results are encouraging. By simple chemical and physical procedures, highly reactive and highly refined preparations have been obtained. It seems likely that such refined products will be less toxic than whole virus for man. If so, the isolated hemagglutinins would be very useful for vaccination, especially of children, because in that age group the rate of febrile response sharply limits the dose of conventional vaccines that is tolerable. These considerations about the future use of purified antigens are at present relatively speculative.

In contrast, a solid body of information and data exists that has been accumulated over the past decade on the efficacy of mineral oil emulsions used in humans as adjuvants in influenza virus vaccines. More than 100,000 doses have been given in field trials carried out under the auspices of the Commission on Influenza during

this period. The results clearly demonstrate that, when proper oils and emulsifiers are used and when small volumes are injected intramuscularly, the oily vaccines are well tolerated and, at a great conservation of antigen, yield highly significant protective effects. For example, the fact that the mineral oil adjuvant Lee vaccine gave moderate protection against influenza B in 1955, while the aqueous Lee vaccine was ineffective that year, has already been mentioned. In the spring of 1958, Lennette[4] observed a protection ratio of 7.1 against Asian influenza in a study carried out at Fort Ord, California. Other trials in the military against Asian influenza, using adjuvant vaccines, have given equally promising results.

When one realizes that antibody levels remain elevated for at least three years after vaccination with mineral-oil–containing adjuvant vaccines (18), and that immunity induced by vaccination correlates well with the antibody levels attained, the extrapolation seems reasonable that we now have at hand a device to obviate the necessity for annual vaccination against influenza. Moreover, other viruses can be readily added to the basic influenza formula. Our current preparation under test contains six strains of influenza and two adenoviruses. Hence, it seems safe to anticipate that adjuvant vaccines will be more widely adopted in the future, as appreciation of their advantages grows.

It is tempting to try to anticipate the trends of the next decade or two, but time is short. Hence, I would like to close with these simple statements: (*1*) The evidence is overwhelming that useful vaccines are now available. (*2*) Further development of more effective vaccines seems inevitable. (*3*) As is evident from many of the presentations at this symposium, our major problem is to make effective use of the tools we have and of those we develop. As physicians, we cannot limit the effort to just counting the dead or studying the fascinating details of the mode of exitus, as was described in yesterday's "chamber of horrors" session. Disease and death can be prevented by vaccination. The time seems over-ripe for optimal application of the knowledge that has accumulated.

[4] Dr. Edwin H. Lennette, Viral and Rickettsial Laboratory, 2151 Berkeley Way, Berkeley 4, California, has kindly permitted the use of his data.

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General Discussion

Dr. C. H. Stuart-Harris: I always tell my students that the history of immunization against influenza really begins with the pioneer work of the Commission on Influenza under, first of all, its illustrious leader, Dr. Thomas Francis, Jr., and now under Dr. Fred Davenport. It might seem that the fact that we thought it worth while in Britain to organize field trials from 1951 onwards was a sign that we were in some way uneasy about American data. This was not true at all for our efforts have been designed merely to widen the application of influenza vaccine by using not only service populations but a population composed of different age groups; also trials enabled experimentation to be carried out with different sorts of vaccines. Now, I would like to run through these English trials very quickly, if I may.

These have been published and I am not going to spend very long on them. The work before the Asian epidemic has already been referred to by Dr. Davenport. We suffered in these earlier trials from a rather low attack rate. These trials were controlled by comparing influenza A with influenza B vaccine; in 1953 we used the Liverpool 1951 strain and obtained a protection ratio of 1.63, which is a reduction in the attack rate of about 40 per cent. A very similar result was obtained in 1956 during which we used industrial workers only. This was a clinical evaluation because we have always taken the view that serologic evaluation suffers from the disadvantage of not estimating the total toll of sickness against which the vaccine was expected to operate. In 1956 also, but at a residential school where there was an intense attack rate in the control group of unvaccinated boys, a much greater reduction in influenza was obtained, much more in line with the Commission's results.

When the Asian virus came on the scene, we had trials organized in public schools, in teachers' training colleges, and also in various miscellaneous centers, mostly with government employees. In this group of trials with the Asian vaccine we did obtain results which were quite concordant with those reported by Dr. Davenport with a fairly good reduction of incidence, providing that one ignored the illnesses in the first week following vaccination before antibodies were fully developed. We did, however, fail to obtain any significant protection with a polyvalent vaccine containing swine, PR8, and FMI, but which did not contain the Asian virus. This has remained a slight bone of contention with us and with Captain Gundelfinger.

Since then we have the results of trials with Asian vaccine in patients with chronic bronchitis who were observed in chest clinics. Again in this group, we obtained results which did not suggest any reduction in terms of illnesses except in the group given Asian vaccine—curiously enough, in male bronchitic patients but not in females. However, the question of diagnosis does arise here because very often influenza in patients with bronchitis looks like an attack of bronchitis anyway, and I think we have some reservations about how the categories of illnesses were devised.

This morning we heard some facts mentioned in the discussions on Dr. Henle's antigens. Now, I am quite bothered about this question of the antibody response to inactive influenza vaccine because, after all, this is the method by which we often select vaccine strains, and the question of potency of the vaccine is evaluated largely on the antibody response. Therefore, in addition, we have carried out a comparison with Dr. Henle's antigens. The V antigens were prepared exactly according to Dr. Henle's formula and tested against sera of immunized animals. They were, as far as can be told, strain-specific V antigens which gave quite sharp specific responses. We have results on fifty cases of influenza which Dr. Hobson[1] has recorded and sent to Dr. Lief. Ninety-six per cent of these showed an antibody response to the soluble antigen. With the hemagglutination test, in these cases of influenza, 44 per cent showed an antibody rise with the Asian hemagglutinin antigen and some also showed a rise in antibody with the other antigens prepared from the various viruses mentioned. With complement-fixation we could not differentiate between the results obtained with any one of these specific antigens, for with each there was a 100 per cent response. But the results with twenty-five sera following influenza vaccination were rather different.

In these instances there were very good responses to a single dose of Asian influenza vaccine given in 1958 as shown by an excellent antibody rise in the ordinary hemagglutination test (92 per cent response). But when we tested the same sera by the complement-fixation test with a purified suspension of the V antigens, we got a 98 per cent response with the Asian strain and only very poor responses (4 to 8 per cent) with the swine and PR8 strains. Therefore, the question which I want to leave with you is this: What is the antibody that we are attempting to measure in the hemagglutination test? Is it the antibody which is the protective one or is it some other?

[1] Hobson, D., and Pearson, E.: Serological response to Asian influenza viruses in man. A comparison of haemagglutination inhibition and complement fixation methods, Brit J Exper Path (to be published in April, 1961).

DR. HARRY M. ROSE (New York, New York): The remarks I have to make deal primarily with a study of immunization against Asian influenza carried out at Fort Dix, New Jersey, in the summer and early fall of 1957, at which time the population was almost entirely at risk, as has already been indicated by the fact that almost no one showed antibodies in his serum to what is now called A2 virus. This study was one of the early efforts to discover something about the protective capacity of a monovalent vaccine given under controlled conditions to a limited number of military personnel.

Between August 2 and September 5, 1957, we immunized three groups of persons. One group (control) was given placebo injections of formalinized salt solution; another was given a vaccine containing 200 chicken erythrocyte agglutinating units per ml.; and the remaining group received a vaccine containing 750 chicken erythrocyte agglutinating units per ml. There were 1,655 persons in the control group, 1,873 persons in the 200-unit group, and 1,665 persons in the 750-unit group. The first column of table 1 shows the number of hospital admissions from each group during the epidemic period which, in terms of this study, was defined as the period between September 20 and October 16. The rate per thousand per week is seen in the next column, and the protection ratio calculated as per cent reduction is seen in the last column. This was approximately 60 per cent for the 200-unit group and 77.5 per cent for the 750-unit group. These epidemiologic data clearly show that both vaccines had a significant protective effect.

Antibody responses to the vaccines were measured by the complement-fixation test, which was performed with "soluble" membrane antigen. The results are shown in table 2. The dates indicated are those on which serum specimens were obtained. The numbers in parentheses are the numbers of men tested in each group. The geometric mean titers for complement-fixing antibody to influenza A and to influenza B viruses are shown in the last two columns. The 750-unit preparation stimulated a fourfold increase in complement-fixing antibody titer within two weeks which remained at about the same level after a further period of two weeks. There was a demonstrable but less marked rise in complement-fixing antibody titer after inoculation

TABLE 1

EFFECT OF MONOVALENT INFLUENZA VACCINES ON HOSPITAL ADMISSIONS FOR ACUTE RESPIRATORY INFECTION DURING EPIDEMIC PERIOD OF ASIAN INFLUENZA

Group	Admissions/ Total	Rate/ 1,000/ Week	Per Cent Reduction
Control	147/1,655	222	—
200-unit vaccine	67/1,873	80	59.9
750-unit vaccine	33/1,665	50	77.5

TABLE 2

COMPLEMENT-FIXATION TITERS OF SERA FROM PERSONS INOCULATED AUGUST 2, 1957, WITH MONOVALENT ASIAN INFLUENZA VIRUS VACCINES

Vaccine and Number Inoculated	Date	Geometric Mean Titers	
		Influenza A	Influenza B
	August		
750 unit (51)*	2	7	6
	16	31	6
	31	28	6
200 unit (52)	2	6	6
	16	15	6
	31	16	7
Controls (47)	2	6	6
	16	6	6
	31	7	6

* The numbers in parentheses indicate the number of men tested in each group.

TABLE 3

HEMAGGLUTINATION-INHIBITION TITERS OF SERA FROM PERSONS INOCULATED AUGUST 2, 1957, WITH MONOVALENT ASIAN INFLUENZA VIRUS VACCINES

(Geometric Means)

Vaccine and Number Inoculated	Date	Antigen			
		PR 301	FM1	A2/ Jap	A2/ Mac*
750 unit (51)†	August 2	20	35	<10	<10
	16	24	41	<10	13
	September 12	26	44	<10	<10
200 unit (52)	August 2	21	49	<10	<10
	16	25	51	<10	<10
	September 12	21	46	<10	<10
Controls (47)	August 2	21	55	<10	<10
	16	20	51	<10	<10
	September 12	19	49	<10	<10

* Inhibitor-sensitive strain.

† The numbers in parentheses indicate the number of men tested in each group.

with the 200-unit vaccine. No changes occurred in the control group and there was no response, as might be expected, to influenza B.

Consider now the results of hemagglutination-inhibition tests on these same sera, as shown in table 3. The tests were run with the FM1, PR301, A2/Japan/305, and A2/McKinnon strains of virus. The McKinnon strain is an inhibitor-sensitive strain of A2 virus which was isolated in our laboratory in January, 1958, and which was selected for these inhibition tests because it was the most reactive strain we encountered among our isolates, not only with convalescent human serum, but also with homologous chicken antiserum. The A2/Japan strain is the egg-mouse-egg-line referred to by others; it is an inhibitor-insensitive strain. The sera were treated with periodate immediately before they were tested. Each vaccine stimulated a slight rise in titer to the PR301 and FM1 strains, particularly noticeable two weeks after inoculation, but there was absolutely no change in titer to the A2/Japan strain. With the A2/McKinnon strain as indicator, there was a barely perceptible response two weeks after inoculation with the vaccine which contained the larger amount of antigen. The controls showed no change in titer.

These results fit very nicely into the frame of reference provided by Dr. Stuart-Harris only a moment ago and raise the question of what we actually are measuring in determining antibody responses to influenza vaccine. It is obvious from the results of the field trials that the vaccines had a protective effect. Yet, if the results of the serologic tests were accepted as the criterion, one might suppose that these same vaccines would probably have little, if any, potency.

I should like to refer briefly to a certain remark made earlier today in connection with antibody responses either in persons infected with A2 virus following vaccination or in persons who had not previously been vaccinated. Our findings are somewhat at variance with those reported by Dr. Jensen. During the period of this study, between August and October, 1957, we were dealing with a population which had not been exposed previously to the infective agent. Among nonimmunized personnel, who developed influenza as proved serologically and by virus isolation, the geometric mean titer of hemagglutination-inhibition tests was 38 in convalescent serum specimens, the acute phase titer being less than 10. At the same time, persons who previously had been inoculated with a vaccine containing 200 chicken erythrocyte agglutinating units per ml., but who nevertheless acquired influenza, had an initial geometric mean titer of less than 10 but a convalescent mean titer of 75, while in those who received the 750-unit preparation, the starting titers were less than 10 and the convalescent titer was 125. It seems clear that immunization provides a stimulus to further antibody development following natural infection.

Finally, I should like to point out that, among the nonvaccinated subjects, only 75 per cent of those having positive complement-fixation tests had positive hemagglutination-inhibition tests whereas, in the vaccinated subjects, all of those with positive complement-fixation tests also had positive hemagglutination-inhibition tests. I state these facts merely to indicate that it remains possible to draw conclusions from serologic data concerning the protective effect of vaccination.

Dr. Jonas E. Salk: My interest in discussing Dr. Davenport's paper arises from the sharing of a conviction that seems to characterize the students of Dr. Francis—a conviction that the knowledge we now possess for the control of influenza exceeds by far the extent to which this knowledge has been applied.

Although there will always be more to be known about any subject, the time does come when enough is known to decide that something can be done to reduce the problem of a given disease. Epidemics recur, and mortality rates rise in excess of the expected, because—for reasons not yet understood—the viruses of influenza have become active. However, when the viruses of influenza become active we, and not nature alone, are in part responsible for the effects thereof because of something we failed to do—and that is, to vaccinate against this disease.

The role of the scientist is to develop new knowledge and to establish evidence in support, or in refutation, of an hypothesis. The force of this knowledge may be sufficient to bring about action, or to withhold action, on the part of those whose responsibilities are in the realm of application of such information. Scientists are sometimes motivated by an existing need, as well as by their curiosity, and are certainly encouraged by the public and by medical and health administrators to bring about means for prevention of disease. It seems that once this is accomplished, however, and the utilization of the newly developed procedure becomes a matter of self-responsibility, or of administrative responsibility, all sorts of reasons are brought to bear to explain away failure to apply the measures of demonstrated effectiveness.

If we dismiss, for the moment, public understanding, which is of ultimate importance, some medical and health administrators and a not small proportion of physicians and scientists seem to stand on the sidelines with implied criticism of limitations of newly developed procedures, waiting for the millenium to come with the ideal. Some seem to "hold the coats" of the laboratory scientists while the battle is fought for the development of

sufficient knowledge and adequate methods for disease prevention. It is now time to reverse the roles and to "hold the coats" for those scientists and administrators of medical and health matters who might take what is known and apply it that we may then know how limited our knowledge really is in coping with the problem of influenza.

Some have the conviction that enough is known to reduce the magnitude of the problem very substantially. Unless the opportunity is provided for study of actual application of procedures now known, what more needs to be learned will never be known. Until this is done, much time, effort, and money is wasted on repetition and on efforts at improving something that may need very little improvement or very little new knowledge. How much of the problem is in the realm of human decision, and how much is in the realm of understanding the natural phenomena involved?

* * *

To keep the basic issues clear it would be helpful to make distinct the component parts of the problem of the immunologic control of influenza.

(*1*) It is generally agreed that effective individual immunity depends upon the presence at the portal of entry of a sufficient concentration of specific antibody. The sufficiency of antibody at the site of invasion depends, in part, upon the level of antibody in the circulating blood and, in part, upon the permeability of the blood-respiratory epithelium barrier; ultimately, it depends upon prior occurrence of natural or artificial contact with the antigen in question.

(*2*) The nature of the quantitative relationship between antibody level and immunity, and the need for high levels of circulating antibody for effective immunity, was shown in studies conducted during the epidemic of influenza A in 1943. In essence, this study revealed that the degree to which the incidence of disease was reduced was related to the degree to which antibody levels were raised in the vaccinated population. It is apparent that, if it had been possible to raise antibody uniformly to the higher levels, the degree of incidence of disease in the vaccinated would have been lowered still further. (In passing, it should be mentioned that, if the proportion of vaccinated persons in a population is increased, a still further reduction can be expected as a result of the herd effect that has been observed in totally vaccinated as compared with partially vaccinated population groups.)

(*3*) The incentive for achieving the highest levels that are practicably attainable is clear. In answer to the question as to how high the level should be, it might be said that the higher the better; the higher the level attained, the greater the persistence at the more effective levels.

The limiting factors in attaining the highest level are clinical and economic. If one eliminates the economic considerations, the upper limit attainable, using a single dose of vaccine, is set by the frequency or severity of systemic reactions that accompany the use of concentrated virus preparations; in infants and young children the "severity" of the reaction is perhaps of greater consequence, and in the adult population the "frequency" of reactions is of first importance. The frequency of reaction rises abruptly when a certain concentration of virus is exceeded; however, the degree of effectiveness in terms of antibody response increases less abruptly. Therefore, the limit of concentration is set essentially by clinical tolerance.

The febrile and other systemic symptoms of vaccine reaction are not due to non-viral impurities but to the noninfectious virus substance itself. When the virus antigen is exposed to freezing temperatures, between 0° and minus 20°C., both toxic and antigenic activities are destroyed, even though the total protein content is not altered. This toxic effect seems to be a biologic property of the virus that is destroyed by freezing, as is the antigenic activity.

(*4*) From the foregoing it would seem that the attainment of high levels of antibody would have to be accomplished, not by indefinite increase in concentration of virus antigen for the achievement of a single-dose effect, but by the use of multiple injections of small quantities of virus at suitably spaced intervals, and/or by potentiating antigenic effectiveness by means of an immunologic adjuvant. It is to be understood, of course, that such procedures would be effective only if the antigenic composition of the vaccine matched or overlapped sufficiently the strains of virus responsible for the subsequent epidemic.

(*5*) The problems posed for the control of recurrent epidemics due to a known virus for which a vaccine could be available is different from those instances in which a significantly new variant has arisen. In the first instance, that of recurrence of known viruses, there is a need for simple measures for potentiating antigenic effectiveness; in the instance of a new antigenic variance, there is a need for measures that would make possible distribution of the limited supply of the new strain to the greatest number in the shortest possible time. The problem is not only that of inducing maximal effectiveness with any vaccine preparation, but of making useful for the greatest number the smallest amount of new antigen, to facilitate the race between man's ability to spread vaccine and the relatively great speed with which the virus spreads.

(*6*) One measure that seemed likely to provide the solution for both problems touched upon above is an adaptation of Freund's work with emulsified

antigens. Friedewald and the Henles reported their experiences showing the advantages and limitations of this procedure. Later, studies on the immunologic classification of polioviruses, using monkeys as the animals that were immunized, reaffirmed the fact that the omission of acid-fast bacilli from the adjuvant mixture still resulted in the induction of highly satisfactory antibody levels without undesirable local side-effects. Moreover, intramuscular injection of the emulsified material appeared to result in distribution of the oily material along the muscle fibers, and this may have contributed to the eventual disappearance of the inoculum after a period of time.

From later studies in human subjects, with emulsified "influenza" vaccines, it was realized that different batches of emulsifying agent (Arlacel® A) contained the variable factor responsible, in part at least, for certain undesirable local reactions. So far as this was traced, it now appears that the variable component was free oleic acid present either because of incomplete esterification in the synthesis of the emulsifying agent (mannide monooleate) or because of de-esterification. In either event, the introduction of an additional purification step produced material essentially free of a toxic factor for mice that was also ulcer-producing when injected intradermally in guinea pigs. The low frequency of undesirable reactions in human subjects, even with toxic emulsifying agents, was sharply reduced further when especially prepared Arlacel A was used.

Two other factors also seem to have been associated with increasing the frequency of the undesirable local reactions when these did occur. First, it was observed that the frequency of reaction was greater in the Negro segment of the population under study. Second, the presence of a high concentration either of viral antigen or of associated nonviral impurities appears also to have been a contributing factor.

The indications from these studies are that it should be possible, through the use of the specially treated emulsifying agent, free of local necrotizing effects, and the use of sufficiently purified antigen, to prepare emulsified vaccines so that the frequency of local cysts is reduced to a point where they may not occur at all, or at least no more frequently nor with greater severity than with the use of alum-precipitated toxoids. Observations have been made of the effects of emulsified vaccines in children who bear the scars of alum cysts and in children in whom alum-precipitated antigens were employed simultaneously. These comparisons, together with the recently increased use of emulsified antigens for treatment of persons with pollen allergies, have further supported the clinical acceptability of the presently available reagents for emulsified preparations.

The extent to which antibody levels are augmented by emulsification, and the extent to which the quantity of antigen can be reduced and still induce levels of antibody of a high order of effectiveness, has been reported repeatedly. The concern regarding the possible occurrence of undesirable local reactions has thus far inhibited acceptance for general use. However, the undesirable local effects observed in the past seem now to be controllable. It is clear that such effects were not an essential concomitant of the desirable immunologic potentiating effect of an emulsified vaccine. It seems that the immunologic advantage can be dissociated from the clinical disadvantages which have occurred.

Concern regarding possible carcinogenic effects that might be attributable to injection of mineral oil, even in such minute amounts as contained in 0.25 or 0.5 ml. of inoculum (50 per cent oil), seems to be without substantial foundation. From the viewpoint of the composition of the oils employed, they are of the straight-chain or paraffinic series of hydrocarbons and possess no cyclical rings; they are free of the kind of fluorescence, in ultraviolet light, associated with carcinogenic hydrocarbons. From the viewpoint of the known clinical or pathologic effects of the chronic use of heavier mineral oils (either as laxatives or for nose-drops) it has been observed in adults who have ingested mineral oil for many years that deposits can be found in the liver and spleen, but pathologic studies do not indicate this is a factor contributing to the neoplastic disease problem. As for the emulsifying agent, it is a substance that has long been used in foods and cosmetics without suspicion of having harmful effects.

* * *

The problem of prevention of influenza persists. The use of mineral oil emulsified vaccine may well prove to be a necessity if, as has long been suggested, the use of broad-spectrum vaccines for influenza should be developed. Mineral oil emulsification furnishes the possibility of approaching the solution by permitting the inclusion of a great many strains in a single preparation for creating a broad effect. When a new antigenic variant appears then, by virtue of the economy of antigen, permissible through emulsification, a much smaller quantity of new virus can be converted into vaccine for more people in an effort to be of some value for the segment of the population not yet affected. The use of such a procedure would make possible the acceleration of the race in favor of man, who would strive to spread vaccine faster than the virus might travel.

The relative advantage of procedures using noninfectious virus for preparing emulsified vaccines

of a newly identified antigenic variant over the possibility for a live-virus vaccine, for which attenuation would be required, is self-evident, especially for attempting to control a newly emerged virulent virus responsible for a rapidly spreading epidemic.

It would seem to be much more hopeful to act upon the hypothesis that, for the most part, influenza can be controlled by immunization and to establish the extent to which this can be accomplished by fully utilizing our present knowledge. It may be found, as has been observed for smallpox, diphtheria and, more recently, polio, that immunization even of a segment of the population will inhibit or limit the development of epidemics and thereby augment protection afforded by individual vaccination. The extent to which reduction in opportunity for viral multiplication will inhibit or limit the development of variants will also, in time, become evident.

Recognizing that: the viruses of influenza were first isolated in 1931 and 1933; the beneficial effect of vaccination was first shown decisively in 1943; the probable usefulness of emulsification of antigens for influenza vaccines was suggested in 1944–1945 and was further extended in the early 1950's; the need for broad-spectrum coverage has been realized since 1947; the widespread epidemics of influenza continue to occur in 1960, Dr. Davenport's analysis demands the most careful attention lest we look back a decade hence and wonder why, when the course of action was clear, so little was done so late.

Dr. Hideo Fukumi: A number of vaccination trials were made in Japan by us at the time of the Asian influenza epidemic, but unfortunately no attempt was made to evaluate the prophylactic effect by comparison with the morbidity rate because of some difficulties. However, we were able to evaluate the immunizing potency of vaccines by serum antibody response after vaccination. Also, we estimated the hemagglutination-inhibition antibody level critical for the risk of A2 infection by the examination of the antibody level at the acute stage of reinfected cases of A2 virus influenza, and some epidemiologic surveillance was done.

Generally speaking, when 0.5 to 1.0 ml. of 300 CCA Miller-Stanley units of A2 vaccine per ml. was employed, the post-vaccinal hemagglutination-inhibition antibody level reached a value of about 1:64 to 1:128, on the average, in those who had had no A2 antibody at the time of inoculation and a value of about 1:256, on the average, in those who had had more or less A2 antibody due to a previous infection.

The examination of reinfected patients revealed that a hemagglutination-inhibition antibody level of about 1:32 was most critical for A2 virus reinfection, and those who had an antibody titer above this level were, roughly speaking, protected from reinfection, though some of them were found to be reinfected. Thus, if we can assume that protection from infection is chiefly afforded by the hemagglutination-inhibition antibody level, whether this is obtained by infection or vaccination, it may be concluded that the vaccines which we used in our study are effective for prophylaxis.

Dr. B. F. Gundelfinger: The antibody response to influenza vaccination by the intracutaneous or subcutaneous route depended on the dose of vaccine administered. The complement-fixing and hemagglutination-inhibiting antibody response to intracutaneous vaccination was superior to that resulting from subcutaneous inoculation when vaccine doses of less than approximately 50 chicken erythrocyte agglutination units were used. At higher vaccine dosages, the subcutaneous route of administration was superior. Since these studies were conducted after Asian influenza had been epidemic in the United States, and since the test groups had measurable pre-vaccination antibody titers to the Asian strain influenza virus, the results undoubtedly represent anamnestic responses.

PROBLEMS

THOMAS FRANCIS, JR. *Chairman*

INFLUENZA VIRUS VACCINE PRODUCTION[1]

I. Wm. McLEAN, JR.

Methods for the manufacture of inactivated influenza virus vaccines have changed very little since the introduction of commercial preparations some fifteen years ago. Problems have been associated usually with the introduction of a new strain and so far these have been resolved without any basic changes in the several processes. Before this audience it would be presumptuous to describe the more or less standard procedures in use, but possibly a few points are worthy of review.

When a major antigenic variant of influenza virus, such as the recent Asian subgroup or the A1 of 1947, makes its appearance, the vaccine manufacturer must determine many characteristics of the new variant before he can commit himself to full-scale production. This developmental work must be done under extreme pressure if vaccine is to be made available in the shortest possible time. Furthermore, unless the manufacturer is fortunate enough to be producing a standard polyvalent influenza vaccine or is engaged in some other program utilizing large numbers of embryonated eggs, the logistic problems of staff, equipment, and materials, particularly egg supply, must be anticipated and scheduled before production of the new strain. In addition to these obvious requirements, the potential manufacturer is confronted with other serious problems which must not be overlooked.

When the new antigenic variant is isolated and recognized as a potential epidemic hazard, it is distributed in this country to interested vaccine manufacturers by the Division of Biologics Standards of the National Institutes of Health. Usually the new strain—or rather strains, since a number of isolates are generally made available—has had no more than three to eight egg passages and is very poorly egg-adapted, particularly for allantoic growth. The first job is to determine optimal conditions for cultivation in embryonated eggs. The age of embryo from ten to twelve days, the temperature of incubation, and the concentration of seed inoculum are but a few of the variables that must be investigated initially and reinvestigated as the strain becomes more egg-adapted. A series of rapid egg passages made at low dilutions alternating with high dilutions is the usual method for selecting virus clones better adapted to growth in the egg. Fluids from individual eggs are tested at each passage and those of highest hemagglutinating activity used for further passage. There is a certain element of luck in this procedure, and sometimes a usable line can be developed in two or three passages or it may take months of work. Fortunately, the manufacturers have been most cooperative in exchanging strains among themselves, and new lines are also obtainable from government, university, and other laboratories. With so many groups on the lookout for a well-adapted egg line, several candidates are usually available within a surprisingly short period.

Freshly isolated strains of influenza virus produce very low titers or nondetectable hemagglutinating activity when tested by the chicken erythrocyte (CCA) agglutination or Miller-Stanley technique specified in the National Institutes of Health requirements. This procedure is much less sensitive than the "pattern test" which is more commonly employed by workers in the influenza field. In fact, there is evidence that the cell avidity of the virus being tested affects the titers obtained by the two procedures in different ways. Thus, there is no absolute correlation between the two tests and, in the course of egg adaptation, pattern titers may rise markedly before appreciable increase occurs in the chicken-cell agglutination level. Good production strains yield 150–300 CCA units per ml. of allantoic fluid and pattern titers in the order of 2,560–10,240. Certain selected passage lines of well-adapted laboratory strains may give very high CCA titers. For example, certain lines of the PR-8 strain yield 500–700 CCA units per ml. It can be demonstrated, however, that these so-

[1] From the Research Laboratories, Parke, Davis and Company, Detroit, Michigan.

called "high lines" are less antigenic on a per CCA unit basis than other passage lines of the same strain of average hemagglutinating capacity. Moreover, our experience shows that such lines are of poor immunizing potency for man as well as for mouse. For practical production purposes under present requirements, we must have a strain producing about 100 CCA units per ml. of crude fluid with an absolute minimum of 50 CCA units per ml. With both the A1 and Asian variants, initial production work was done with strains producing only about 10 CCA units per ml. of fluid. It is noteworthy, however, that inactivated vaccines concentrated five or ten times prepared from these early passage isolates gave good response in animals and are comparable with vaccines prepared from selected later passage material at a much higher CCA unitage. From electron microscopic studies and other data it appears that the number of virus particles and antigenic mass per CCA unit decreases as the strain becomes better adapted to the egg.

The next problem for the vaccine producer is to determine if the new strain can be concentrated and purified by his standard procedure. This of course requires production of reasonable volumes of crude fluid for trial runs and must await the results of the early passages. The popularity of the Sharples centrifuge method, which is used with various modifications by the majority of the producers, is due most probably to its adaptability in handling new strains. Precipitation and adsorption-elution methods require time-consuming experiments to set up optimal conditions for the new strain. Furthermore, one must continually be alert to the possibility of need for modification of the process, since the properties of the strain may change during the course of increasing adaptation to the egg. Centrifugation procedures, on the other hand, require neither modification for the different strains nor elaborate process controls and adjustments from lot to lot of crude fluid. A process that is not sensitive to subtle changes in the properties of the virus has a distinct advantage since several months to a year are required before a reasonably reliable, stable production line of a new strain can be developed.

Conditions for proper inactivation are the next problem. Most of the manufacturers use formalin in various ways within the limits set by the requirements. It is permissible to use up to 0.2 per cent formalin on concentrates, but the final vaccine can contain not more than 0.05 per cent. The higher levels must be used with caution since they may be deleterious to both hemagglutinating activity and antigen. Early A1 isolates, for example, were very sensitive and lost both hemagglutinating activity and antigenicity even when treated with minimal formalin concentrations. As a result we switched to ultraviolet inactivation of our influenza vaccine at this period. However, more formalin-stable A1 lines were soon found which were used by the other manufacturers with no difficulty. On the other hand, when the Cuppett strain was substituted for FM-1, we found that with this strain the ultraviolet-irradiated virus concentrate lost potency during storage unless a very low concentration of formalin was added to act as a stabilizer. The early Asian strains were not sensitive to formalin, and to my knowledge no difficulty has been encountered in their inactivation. However, it is impossible to predict how a new strain will react to formalin and one must be prepared to modify procedures as required.

Next is the problem of potency. As mentioned above, it is difficult to evaluate the potency of a new influenza variant on the basis of CCA activity. There will be marked variation between different passage lines of the same strain until the lines become stabilized in eggs; and the ratio of hemagglutinating activity to antigenicity will also be a variable factor. Thus, CCA activity is not a reliable guide to antigenic potency in the early stages of egg adaptation. It would be best, when a major variant strain is introduced, to require the vaccine to be concentrated at least five times with respect to the crude allantoic fluid and not attempt to establish CCA requirements. Later when stable, adapted lines are available, a meaningful CCA concentration factor can be established based on animal studies and clinical trials.

At present, formal potency requirements under the standard mouse test are set up by the Division of Biologics Standards as soon as a mouse-adapted line of the new antigenic variant becomes available. This introduces another problem for the manufacturer. Will his carefully selected, egg-adapted production seed line give satisfactory results when tested against the mouse-adapted divergent passage line distributed as challenge virus by the Division of Biologics Standards? Usually he will find it to his ad-

vantage to use the challenge virus line readapted to eggs for his production virus. This change of production lines again necessitates a series of tests before standard production can be resumed. It seems to me that this problem could be avoided if a minor modification in the present potency requirements were made: Allow the serum-neutralization part of the present procedure to be done in embryonated eggs instead of by intranasal inoculation of the test serum-virus mixtures into mice. This is not a new suggestion, but is certainly worth reconsideration at this time.

In conclusion, I would like to emphasize three points: (*1*) It takes time to egg-adapt and stabilize a new antigenic variant of influenza virus for vaccine production. Therefore, early production lots should be considered as emergency experimental materials. Production standards for the new strain should be defined only after laboratory lines are stabilized and the properties of the virus established. (*2*) Only if a potential producer of emergency supplies of a new antigenic variant influenza vaccine has an established and functioning egg facility and at least the nucleus of a trained staff, can he be expected to produce significant amounts of vaccine in less than four to six months. (*3*) Unless this potential producer of vaccine has had some experience in handling eggs in quantity and has worked on influenza virus vaccines, he will be lucky to have supplies of usable product within twelve months.

SOME PROBLEMS IN THE STANDARDIZATION AND CONTROL OF INFLUENZA VACCINE IN 1957

RODERICK MURRAY[1]

Introduction

Dr. Davenport has already given a detailed account of the theoretic and practical basis for the formulation and use of influenza vaccine and Dr. Francis in his address has covered a number of the important problems in the preparation and use of the vaccine, including that of the basis for providing broad antigenic coverage by a polyvalent vaccine. The situation in 1957 presented problems, some of which actually defied solution, merely because of the size and scope of the programs in influenza vaccine manufacture and use. Not the least of these, and outside the scope of the present report, was the continuing economic problem of who would underwrite the cost of expanding production. Never before had there been such a large demand for influenza vaccine. In the circumstances, it was impossible to provide the well-balanced polyvalent vaccine which experience and theory called for, since to produce such a product would seriously limit the availability of antigens for use in immunization against the Asian strain. This report will cover briefly some of the problems encountered by a national control agency in this situation. The real control problem encountered was the development of requirements, particularly for potency, under conditions of extreme urgency at an early stage, in the absence of very much definite information.

It is true that this was not a new vaccine. There were certain new features to the vaccine, but the basic principles concerned with this type of influenza vaccine had been well worked out since influenza vaccine was first introduced as a licensed product in 1944. At the time of the appearance of this new variant, there were six firms licensed under the Public Health Service Act engaged in the manufacture of influenza vaccine. Five had been licensed in 1944 and the sixth, in 1947. A seventh producer was licensed late in 1957, but this was too late to make any contribution to the national needs for vaccine for that season.

Decisions as to whether vaccine should be produced in the normal way or whether it should be manufactured on a high national priority using every conceivable facility would seem to depend in some measure upon the evaluation of the immediate clinical severity of the illness caused by the particular epidemic strains. Consequently, early health intelligence reports were carefully scanned to determine whether there was any unusual clinical severity and whether there were any unusual features which were at variance with past experience, such as different age group differentials. Always before the minds of those concerned at this stage was the picture which has been permanently impressed upon us by the great pandemic of 1918, although early reports, as Dr. Francis discussed last evening, indicated that this would probably not be repeated. Although there were no formal decisions made concerning these matters, there seemed to be general acceptance of the principle of all-out production using existing and perhaps expanded facilities in being. What the national effort would have been had the clinical manifestations indicated a possibility of a repetition of the 1918 experience can only be guessed at.

In order to make available the greatest quantity of vaccine at the earliest possible time, requirements were modified whenever it was possible to do so and still maintain the safety, purity, and potency of the product. In the interest of expediency, potency was determined on the basis of chicken erythrocyte agglutination (CCA) content[2] and the requirement for a mouse antigenicity test, which required four or more weeks to complete, was temporarily suspended. Arrangements were made whereby concurrent safety and potency testing would be done by the Division of Biologics Standards and the manufacturers. The manufacturer forwarded samples of each lot of vaccine to the Division for testing at the same time his own tests were initiated. Later when the producer had obtained satisfactory test results in his own control laboratory, he submitted to the Division a com-

[1] Division of Biologics Standards, National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare.

[2] An expedient resorted to again in January, 1960, when there were increased needs for vaccine.

pleted protocol and a formal request for the release of the specific lot of vaccine. In this way no time was lost in effecting the release of lots of vaccine because of the testing program of the Division of Biologics Standards. On some occasions, the Division, having already completed satisfactory tests of its own, was able to clear a lot for release the same day the manufacturer's protocol was received. During September and October, when the pressure for vaccine was greatest, approximately 45 per cent of releases were effected by the Division within twenty-four hours of the receipt of the finished protocols. The first vaccine was cleared for release by the Division on August 12 and, by the end of August, a total of 2,500,000 ml. had been cleared.

Production Problems

The main initial manufacturing problem encountered with the Asian strain influenza vaccine was the selection of a particular isolate which could be adapted to produce satisfactory virus growth in the chick embryo. The early experience with each of the isolates available was not very satisfactory. The allantoic fluid harvested after virus growth showed a CCA titer as low as 20 in some instances. Normal manufacturing practice requires a much higher titer, preferably in the hundreds. Despite these low titers, the manufacturers believed that they could prepare a vaccine, albeit at considerable cost. It was estimated that one dose of vaccine containing 200 CCA units of antigen could be produced per egg. A number of these strains eventually became well adapted to growth in embryonated eggs, and the yields were considerably higher. As Dr. McLean has pointed out, the possibility exists that adequate amounts of virus may actually be present in the harvested fluids and that the initial low CCA values are really testing artifacts. This is a retrospective deduction but could perhaps be a fit subject for future study. It does illustrate the great need for means to measure precisely a number of properties in a new variant. The real estimate of potency for a new variant, as will be indicated later, is the determination of antibody response in man and the correlation of this response with laboratory tests of the vaccine.

A most important matter affecting the national ability to produce vaccine is the availability of suitable embryonated eggs. Normally this is a matter of considerable advance planning between the manufacturers and the poultry industry which, geared to the production of poultry for human consumption, has a seasonal variation in the availability of embryonated eggs. Fortunately the supply was adequate in 1957.

The Role of the National Institutes of Health and the Division of Biologics Standards

The United States statute which governs the control of biologic products stipulates in effect that biologic products as defined may not be sold, bartered, or exchanged unless manufactured in a licensed establishment. Regulations relating to the standards applicable to such biologic products are promulgated by the Surgeon General of the Public Health Service. In effect, the Division of Biologics Standards administers the control of biologic products and is responsible for initiating action in the adoption of standards, regulations, et cetera, when these are required to assure the safety, purity, and potency of biologic products. It maintains licensing, inspection, and control testing programs for biologics and conducts research relating to the development, testing, and use of such products. The Division was active from the beginning with respect to Asian influenza virus and was able to provide strains for the manufacturers and others interested at a very early date. It also provided the manufacturers with available information with respect to the various strains and made proposals concerning potency based on the information available.

Each of the licensed manufacturers had a long experience in the manufacture of the product of ten or more years. The year 1957 was not the first instance in which an attempt was made to initiate production with a new strain. It happened in 1951 when the "London" strain was isolated. The strain was sent out to each of the manufacturers licensed at that time and they were able to produce token quantities of vaccine in thirty to sixty days. Fortunately, that particular strain did not give rise to a widespread epidemic, and the manufacturers were not required to implement their strategic exercise.

Testing Program

The chicken erythrocyte agglutination test: The chicken erythrocyte agglutination test is normally applied to determine the virus content or antigenicity at various stages of production

and as a guide in pooling to form polyvalent vaccines. In order to have comparable and standardized values, the National Institutes of Health have for a number of years recommended that the CCA values be determined by one method, that of Miller and Stanley.[8] The Division of Biologics Standards provides a reference vaccine for use in the standardization of this test. Since widespread use of this reference vaccine would quickly deplete supplies of a valuable reference material, manufacturers usually prepare a working standard or reference of their own and compare this with the National Institutes of Health reference vaccine for production purposes.

Test for inactivation: Each lot of vaccine is tested for freedom from viable influenza by the intranasal instillation of 1 ml. in 0.05 ml. amounts into young Swiss mice. The animals are observed for ten days and examined for specific pulmonary lesions. Since the Asian strain as used for production had not been adapted to mice, an additional test for inactivation by the inoculation of eggs was performed. The view is often expressed that, since it is highly unlikely that any influenza virus remains viable after the inactivation process and, if so, it would be present in such small amounts as to be incapable of causing infection, the performance of these tests is academic. Whatever the merits of this argument, it is wise to test each lot of vaccine for freedom from influenza virus as a measure of determining the effectiveness of the inactivation method.

Sterility tests: These tests are carried out to control the problems of bacterial contamination and are performed at a number of points during processing. A required test is the final container sterility test which is performed at two distinct incubation temperatures: one, at 32°C. and the other, at approximately 22°C. The latter is to detect organisms which fail to grow at 35°–37°C., which may be missed at 32°C., but which grow rather well at 22°C. and represent a contamination problem in products derived from embryonated eggs. Contaminants of this type are usually gram-negative rods and have often been implicated in pyrogen problems encountered in the manufacture of biologic products.

Potency tests in mice: The potency test which is usually performed on influenza vaccines is the mouse protection type and involves the inoculation of groups of mice with graded dilutions of vaccine. After fourteen days the mice are bled and the serum pooled according to groups. The antibody content of each pool is then determined by mixing dilutions of the serum with a homologous virus strain which is lethal for mice. In this way protection can be demonstrated against the virus according to the dilution series of the vaccine. Apart from delays in performing the mouse test itself, it was not possible to perform this test with Asian strain vaccine in mid-1957 because of the time required to develop a strain lethal to mice in sufficient titer to be successfully used in such a test.

[8] Journal of Experimental Medicine, 1944, *79*, 185.

Testing Program at the Division of Biologics Standards

While it is clear from the statute and the regulations that the ultimate responsibility for the safety, purity, and potency of the products which they produce rests with the manufacturers, the Division of Biologics Standards engages in a program of testing influenza vaccine from a dual point of view: to determine whether the required standards are being met by the manufacturers, and to develop information which will lead to better testing methods and thus assure improvement of the product. The following tests were carried out on Asian strain influenza vaccine by the Division:

(*1*) Chicken erythrocyte agglutination determinations on the final product. This index is subject to variation with the passage of time, but it is anticipated that valuable information will be forthcoming in the collection of these data.

(*2*) Mouse inactivation tests. These were carried out on the first five lots produced by each manufacturer. Thereafter they were performed as indicated.

(*3*) The mouse potency test, although not required initially for release of Asian strain influenza vaccine, was performed as soon as feasible on a number of lots from each manufacturer for the purpose of collecting data.

(*4*) Sterility tests.

(*5*) Safety tests in mice.

In addition to the tests mentioned, the Division also investigated the problem of pyrogenicity of some vaccines. While some were actually pyrogenic, considering the manufacturing process employed, a surprising number were free of pyrogens as determined in the standard pyrogen test.

A problem of increased toxicity in the so-called innocuity test performed on final container material with some 1958–1959 lots of 1,000 CCA unit vaccine remains unsolved, but this toxicity was not reflected on injection of the material into human subjects.

At the time the Asian influenza strain emerged, two formulas for vaccine were available. These are given in table 1.

With the appearance of the Asian strain, manufacturers were advised on July 2, 1957, that three vaccine formulations would be acceptable. These are listed in table 2. These proposed formulas were arrived at after consultation with a group of advisors and represented the best thinking at the time. There was no experimental evidence at the time to indicate what amount of Asian strain antigen was necessary to provide protection.

As is now well known, it soon became apparent that production of polyvalent vaccine, while advisable on theoretic grounds, had the great practical disadvantage of reducing the number of doses of vaccine containing Asian strain material which would become available. The Public Health Service and other interested groups soon accepted the principle of using a monovalent vaccine containing only the Asian strain as the best means of assuring adequate production and supply of vaccine to meet the threat of an impending epidemic. Accordingly, on July 26, 1957, the manufacturers were advised that a monovalent vaccine containing 200 CCA units would be acceptable for both civilian and military use. The choice of the figure of 200 for the CCA unit content of each of the formulas mentioned and for the proposed monovalent vaccine was based mainly on past experience with other variants. However, preliminary information from work being conducted by Bell, Jensen, Rose, Meiklejohn, and others indicated that this was not a bad estimate.

The early need for decision concerning potency requirements illustrates perhaps the most important information needed from the control point of view: early, precise information concerning the amount of antigen needed to produce adequate and, hopefully, protective levels of antibody. Early information on this point is imperative, and critical studies designed to obtain this information should be given top priority as soon as the earliest experimental batches are available from any manufacturing source. Use of this valuable early material for the vaccination of self-appointed recipients and "V.I.P."'s is to be deplored.

As additional information on antibody response and protection became available during September, 1957, it appeared that the 200 CCA unit figure could be improved. An ad hoc committee which met at the National Institutes of Health on September 26 advised as follows:

The Committee has reviewed the data dealing with serologic responses and protective effects elicited in man by Asian monovalent influenza vaccine having an antigenic potency of 200 CCA units. The recent information, like that obtained earlier, indicates that administration of 1.0 ml. subcutaneously of a vaccine of this potency is of definite value. Thus, it is capable of eliciting detectable

TABLE 1

COMPOSITION OF INFLUENZA VACCINES FOR CIVILIAN AND MILITARY USE, 1957

Strain	Number of Chicken Erythrocyte Agglutination Units	Use
Swine 15-1926	111	Civilian
PR-8	111	
PR-301	111	
Lee	83	
Great Lakes 1739	83	
Total*	500	
Swine 15-1926	200	Military
PR-8	200	
PR-301	200	
Lee	200	
Great Lakes 1739	200	
Total	1,000	

* Approximate

TABLE 2

COMPOSITION OF VACCINES CONTAINING THE ASIAN STRAIN

Strains	Number of Chicken Erythrocyte Agglutination Units	Use
A. Monovalent	200	
B. Polyvalent		Military
Swine 15-1976	200	
PR-8	200	
PR-301 (or a 1957A1)	200	
Great Lakes 1739	200	
Asian strain	200	
Total	1,000	
C. Polyvalent		Civilian
Swine 15-1976	75	
PR-8	75	
PR-301 (A.A.1957A1)	75	
Great Lakes 1739	75	
Far East	200	
Total	500	

antibodies in a majority of persons and presumably of protecting an appreciable proportion of those vaccinated against clinical disease. However, when 400 CCA vaccine is administered, antibodies develop in more people and to a higher level. On the basis of all previous information this should induce more effective immunity more promptly. Indeed, the antibody levels in such persons are comparable to those in patients who have recovered from Asian influenza. Because of the intense efforts of the vaccine manufacturers, production methods and facilities have improved to the point where 400 CCA vaccine might be made available for general use in the near future.

In view of this, the committee made the following recommendation:

That the antigenic potency of Asian monovalent influenza vaccine be increased from 200 CCA units per ml. to 400 CCA units per ml. as soon as feasible and not later than December 1, 1957.

After reassessing the capabilities of industry to meet this recommendation, all manufacturers were advised that beginning December 1 monovalent vaccine would be considered for release only when containing a minimum of 400 CCA of the Asian strain per ml. Actually 400 CCA unit material began to be released in the first week of November.

By November it was possible to consider returning to a polyvalent vaccine. This was made possible by the fact that industrial production was beginning to outstrip requirements for vaccine. Moreover, it was considered imperative, in view of the fact that outbreaks due to other strains might occur later in the season or in the following year. The military polyvalent vaccine proposed was one containing 1,000 CCA units per dose, of which 400 would be represented by the Asian strain. This presented no serious problems in formulation or manufacture because the remaining 600 units could be comfortably distributed between the other antigens. The civilian formula with its upper limit of 500 CCA units, based upon the experience that people show a disinclination to accept the local and general reactions which sometimes follow the administration of more than this amount of antigen per dose, presented a serious problem if a dose of 400 CCA units of the Asian strain were actually needed. This matter was given careful consideration by a group of advisors, and their recommendation, which was subsequently formalized in a communication to the manufacturers, was for a polyvalent vaccine, as shown in table 3.

TABLE 3

POLYVALENT VACCINE CONTAINING THE ASIAN STRAIN

Strain	Number of Chicken Erythrocyte Agglutination Units
Asian	200
Great Lakes	100
PR-8	100
PR-301	100

On paper this would look like a reversion to a dose of 200 CCA units in the case of the Asian strain. However, the dosage recommendation called for a change from a single dose to a series of two doses given not less than two weeks apart in the case of persons who had had no previous immunization with Asian strain material. Such a polyvalent vaccine became available during January, 1958, but by this time the tremendous demand for influenza vaccine of any formulation had abated.

The only change in the formula of the vaccine which has been made since that time has been the substitution of the Ann Arbor 1/57 strain for the PR-8, since the former seemed to be superior as an antigen. The experience with the exacerbation of influenza in the winter of 1959–1960 is not such as to require any changes in the formula.

Production Experience in 1957

A rather clear picture of the course of the production program is given in figure 1. It indicates the relatively large effort made by industry and the extent of the achievement. All of the licensed manufacturers had had a great deal of experience over the years and had previously met and solved many of the problems which confronted them in 1957.

Isolates of the Asian strain virus became available from Walter Reed Army Institute of Research in May and were provided to the various licensed manufacturers on May 12, 1957. Between May 20 and May 31, several informal discussions were held by members of the National Institutes of Health, the Office of the Surgeon General, and the military services relating to possible courses of action and anticipated problems.

By mid-June small quantities of Asian strain vaccine had been prepared by some manu-

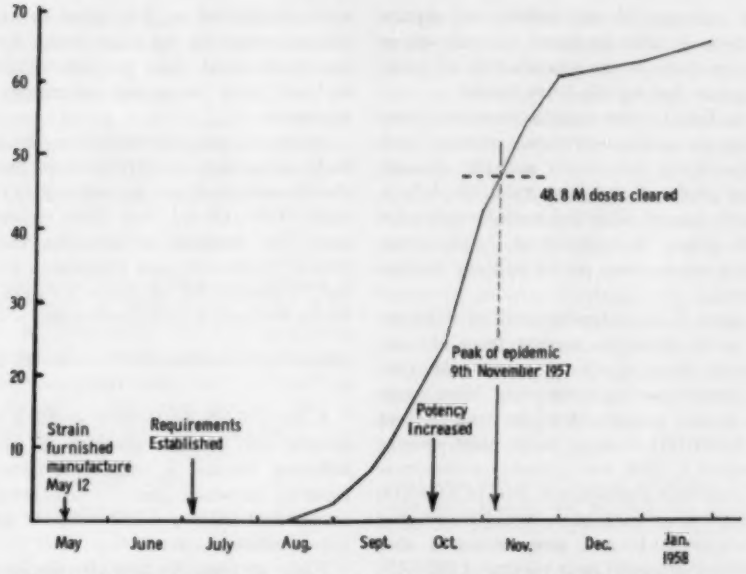

FIG. 1. Asian strain influenza vaccine cleared for release (millions of milliliters).

facturers and had been provided for experimental use. On June 12 a meeting with technical representatives of the six licensed manufacturers of biologic products was held at the National Institutes of Health. At this meeting there was a free exchange of information on the strains available and on their growth characteristics. Following this, plans were made by all manufacturers for maximal production of the new vaccine in order to meet the anticipated emergency requirements. The manufacturers also began assembling data for the Division of Biologics Standards on production capacity. Study by the Division of these reports in the light of the virus yield then being obtained indicated that approximately 3,000,000 doses of monovalent Asian strain vaccine could be produced each week provided there was no concurrent production of polyvalent vaccine. This level, it was estimated, could be reached without marked changes in plant policy or operation. Under conditions of a true national emergency, probably at least 5,000,000 doses of monovalent vaccine could be produced each week, but this would require major policy decisions by the management of each of the licensed producers. If the questions of low potency and poor growth of the virus, which continued to be a matter of primary concern to all producers, could have been solved, it was conceivable that the elimination of these obstacles alone could double the output to approximately 10,000,000 doses per week. By the end of June most of the manufacturers were either in the process of increasing production of Asian strain material to the limit of their capacity or of tooling up for this eventuality. It was clear to all concerned that adequate production of potent vaccine for large-scale immunization was not to be anticipated within the next month and that even reasonable amounts would not become available before two months. It should be noted that, when local epidemics of Asian influenza first appeared in the civilian population of the United States in late June, the manufacturers had already been working with this strain for approximately six weeks.

The initial manufacturing problem was low virus yield, and there were some initial reports that various isolates of the Asian strain gave better yields than others. By mid-July it appeared that the problem of low virus yield was being solved, the average yield reported by the producers at this time being approximately five times that initially obtained. By the end of July, higher yields were being consistently reported by all manufacturers, and there had been con-

siderable exchange of information and strains among them. It thus appeared that the major technical problem in the manufacture of Asian strain vaccine was rapidly being solved.

Prior to July 1, most manufacturers had been doing only the preliminary developmental work required with the new strain, and the amounts of vaccine produced had been small. On July 2, orders were placed with the manufacturers for 2,650,000 doses of monovalent Asian strain vaccine for use by some of the military services (*see* figure 1).

By August 1, all manufacturers of influenza vaccine were definitely moving from the developmental stage of operation into the production phase involving monovalent Asian strain vaccine. It now appeared that the originally set goal of 60,000,000 doses of monovalent vaccine by February 1, 1958, was probably a conservative one, and that a production level of 5,000,000 to 7,000,000 doses each week would be reached by mid-October. It was now estimated that 85,000,000 ml. of monovalent vaccine of 200 CCA units could be produced by January 1, 1958. It might be noted in passing that at the Special Conference on Influenza held by the Surgeon General with state and territorial health officials it was estimated that 60,000,000 doses could be released by February 1, 1958. Actually, this figure was exceeded by January 1.

By mid-November, 6,000,000 ml. of 400 CCA unit material had been cleared by the Division of Biologics Standards for release and no new production of 200 CCA material was being initiated. The total quantity of Asian strain vaccine (both 200 and 400 CCA material) cleared by this date was 53,000,000 ml. (*see* figure 1).

Polyvalent vaccine: Although polyvalent vaccine had always been recognized as the influenza vaccine of choice, the Public Health Service originally accepted the principle of a monovalent Asian strain influenza vaccine in the belief that this was the best means of assuring adequate production of vaccine to meet the threat of a possible epidemic during the fall and winter months. By mid-November, 1957, it was apparent that the demands for monovalent vaccine would be met soon and that, with the immediate emergency covered, the manufacturers would soon begin to cut back production. With a view toward focusing attention on the manufacture of a broad-coverage polyvalent vaccine before production momentum was lost, requirements were established at this time for polyvalent vaccine containing the Asian strain. At this time the requirement that potency determinations be based upon the mouse antigenicity test was reinstated.

Decreasing demand for vaccine: Production of both monovalent and polyvalent Asian strain vaccine continued and by January 1, 1958, more than 61,000,000 ml. had been cleared for release. The demand for influenza vaccine continued to decrease, and production was accordingly curtailed by all manufacturers, some of whom now had sizable inventories.

Reported Reaction to Penicillin in Influenza Vaccine

A particularly nettlesome problem arose concerning the possible presence of penicillin in influenza vaccine. A number of instances were reported in which penicillin-like sensitivity reactions had developed following the administration of influenza vaccine.

While no penicillin was actually added to the vaccine itself, traces of penicillin were present in some of the seed virus which had been used in production. The amounts involved were considerably below levels which could be determined by any test, and calculations based upon dilution, washing, et cetera, indicated that the amounts must have been less than 0.001 units of penicillin per dose of final vaccine. Investigations of the vaccines used in 4 reported cases of sensitivity reactions indicated that in three of the four, the vaccines did not have penicillin even in the seed virus stage. Thus, the reactions must have been due to some other cause. However, since the physicians involved felt rather strongly on this subject, and since a contention was made that even as little as 0.0005 units of penicillin would be sufficient to produce a reaction in an exquisitely sensitive individual, all manufacturers agreed to abandon the use of penicillin in their seed virus.

Dosage Recommendations

One very confusing feature encountered in the control of the vaccine in 1957 concerned dosage recommendations. The recommendations of the Public Health Service, the American Academy of Pediatrics (except in regard to very small children), and other bodies were very clearly based upon subcutaneous or intramuscular inoculation of the vaccine. This was also

true of the recommendations contained in the package enclosures provided by the manufacturers. Nevertheless, a number of physicians chose to administer fractions of a milliliter intracutaneously, basing their decision on results which had been obtained with earlier strains in *post*-epidemic periods. This is a practice which should be avoided if possible in the future.

Need for New Methods

The quantitative measurement of antigens is notoriously imprecise and results vary from laboratory to laboratory. We need improved and rapid methods of measuring influenza virus antigens in vaccines. This is not very exciting research during interepidemic periods, but is nevertheless one of our great control needs.

Summary

Although a number of problems were encountered in 1957, these were overcome by the combined efforts of research workers and the excellent cooperation of the manufacturers. The outstanding feature of the experience has been the demonstration that, even with the comparatively tremendous lead time available in the United States and the prodigious performance of biologic producers, not enough vaccine was available soon enough to meet our needs.[4] This problem will undoubtedly arise again even more acutely and we must have an answer. Whether the answer is the use of adjuvants, some of the methods which have been suggested today by Dr. Davenport, the use of live vaccines, the development of different methods of administration, or something else, remains to be seen. Certainly the use of larger chicken erythroycte agglutination unit content, while necessary to assure protection to particular individuals, is not a helpful course on a national scale. The problem of getting more "mileage" out of available antigen requires urgent investigation.

[4] If the curves of excess mortality shown by Langmuir, Dauer, and Serfling are superimposed upon the chart of production (*see* figure 1), it will be seen that the peak of the epidemic occurred on November 9, 1957, at a time when 48.8 million doses had been cleared for use. If one week is allowed for entering vaccine into distribution, only 42 million doses were available by the time of the peak of the epidemic.

PROBLEMS OF ALLOCATION AND DISTRIBUTION OF INFLUENZA VACCINES[1]

DORLAND J. DAVIS

Discussion of the allocation and distribution of influenza vaccines assumes that we have an effective vaccine for allocation. This, I think, is a good assumption on the basis of what we have heard so far in this conference, and on the promise of improved products to come.

Another assumption, much more tenuous, is that an epidemic can be fairly well predicted before the time for effective vaccination. This takes into account an increased ability to forecast the course of epidemics. We are in a better position to do this now than ever before in the many years that epidemics of influenza have afflicted mankind. For example, the 1957 epidemic in the United States and Europe was quite accurately forecast in late May and early June of that year. The subsequent course of events followed very closely the predictions of experienced epidemiologists and the advisors of the Public Health Service. Similarly, this last fall the Public Health Service ventured the general forecast that there would be scattered outbreaks of influenza this winter. This has turned out to be the case. I think that we should be confident that we are in an increasingly favorable position to forecast the occurrence of the disease.

The problem of distribution of vaccines during the years of lesser epidemics and outbreaks of influenza A, such as occurred in 1951, 1953, and the current year, 1960, poses one type of problem in the area of distribution and allocation. In general, distribution and allocation are determined by the normal demands of medical and public health practice. The supply is scheduled to meet a base annual requirement of the military services and the anticipated demand from civilian practice.

Unfortunately, the heaviest civilian demand usually comes when cases begin to occur and are reported in official documents and in the press. As we all know, vaccine must be administered about a month prior to the peak of an epidemic. Vaccine given during the course of the epidemic can have little or no effect in reducing incidence except among the relatively small number of persons who would have acquired their illness at the end of the epidemic period. As individuals, these persons are likely to be the chronically ill and aged who represent special risks. Vaccination of these persons is worth while, but we should expect very little, if any, discernible effect from vaccination during an epidemic.

An urgent problem is the allocation and distribution of vaccines which may be in short supply prior to a pandemic caused by a new variant strain. The first decision to be made is to whom this meager supply of vaccines should be given.

One criterion for this decision would be based on the prevention of death, the most serious complication of influenza. The 1957–1958 experience indicates that there were some 88,000 total excess deaths from October, 1957 to March, 1958 (1). At least one-half of these were in those aged sixty-five and older. Of this number, 20,000 deaths were ascribed specifically to influenza and pneumonia, more than 50 per cent of them occurring in those sixty-five years of age or older.

In the United States there are now approximately 15 million persons more than sixty-five years of age. The most recent National Health Survey figures show that, of these, 2,063,000 suffer from disabling chronic conditions (2). Another figure from the same source indicates that approximately one million of those more than sixty-five suffer from asthma and chronic bronchitis. Thus, there is a significant number of persons at high risk to an attack of influenza in the older age group.

If a vaccine priority were to be based on preexisting illness *for all ages*, the numbers would be of significant magnitude. For instance, the recent National Health Survey indicates that there are 8,103,000 persons with asthma and an additional 2,127,000 with chronic bronchitis, totaling more than 10 million of all ages (2).

Previous estimates indicate that there are 10 million people with cardiovascular-renal disease in the country and approximately one million with known diabetes. The last epidemic

[1] From the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland.

indicated that pregnant women were at an increased death risk. With the present birth rate, we can estimate that there are at any one time 3.5 million pregnant women in the United States.

Thus, it appears that, if a priority is based upon age, pre-existing disease states, and pregnancy, the number of persons deserving vaccination will be in the order of 30 to 35 million.

In the second general category which deserves protection in the face of an epidemic are the medical personnel who will have the responsibility of the care and treatment of the ill. These now number approximately 1.5 million. In general these are young adults who usually have a low risk of fatality, but whose services are essential to cope with the problems of an epidemic.

The third category would cover those in essential industry. Persons engaged in communication services, utility services, and police and fire protection number approximately 1.6 million. To these may be added those in transportation services, comprising 3 million persons. It is assumed that the military population will have been immunized under a continuing program of high priority to their needs.

If supplies are sufficient, the problem of immunizing the priority groups will depend upon the leadership and guidance of the medical and public health officials and the public's acceptance of vaccination. If the supply of vaccine is as limited as it was in 1957, the problem of distributing it to those most needing protection becomes acute and of public concern. For instance, by September 5, 1957, only 4.6 million milliliters of vaccine were available. This was the time, about four to six weeks before the height of the pandemic, when the vaccine would have been of maximal effectiveness.

Recent reports have confirmed earlier observations that intradermal injection evokes an antibody response (3, 4), and this conference perhaps will develop data on the actual effectiveness of disease prevention by vaccine given intradermally. If this proves to be a satisfactory method, it would increase the available immunizing doses by a factor of 5 to 10, because fewer chicken erythrocyte agglutination (CCA) units are ordinarily used. The 4.6 million milliliters ready at the critical time in 1957 would have been sufficient for 23–46 million doses.

The foregoing plan of allocation first attempts to protect those who are at greatest risk of death and next, those whose incapacity may disrupt normal community activities.

Another approach would be to attempt to prevent infection among those most likely to spread the disease in the community. Epidemiologic studies of epidemics of influenza show that the highest age-specific attack rates occurred in the school age students (5) and that these students may constitute the most important source of infection to the family. This concept is further supported by the observation of a delay in the occurrence of deaths following the peak of morbidity. It suggests that those at greatest death risk acquire their infection later than more active people such as school children.

The question might be raised: Why not vaccinate all high school and elementary school students? As an important source of infection to their parents, those chronically ill in their homes, and infants, they may form a vulnerable link in the chain of contact. However, there are now approximately 42,700,000 children in school (kindergarten through twelfth grade) in the United States, so the vaccine supply would have to be adequate and available well before the anticipated epidemic. One would hope, nevertheless, that the opportunity would be afforded sometime to evaluate such a method of immunizing those who are important in the spreading of the infection.

We might look for a moment at the experience of 1957–1958 in distribution and allocation of vaccines. At that time, you will recall, the manufacturers agreed to distribute vaccines to the several states in proportion to the population of the state to that of the nation. The management of the distribution within the state became the responsibility of the medical profession, the state health officers, and regular distribution channels.

This seemed at the time the only practical way and, unless a continuing program is maintained, it might be the only course open again. That this was less than satisfactory was pointed out by numerous instances of uncontrolled distribution and a number of statements by members of the medical and health service professions.

In many instances recommended priority groups will be ignored in favor of groups more accessible and important to those administering the vaccine.

On a national level, the Public Health Service can advise those directly responsible for vaccination on the categories that should have priority

for the limited supply. It can assume leadership in working out uniform procedures for appropriate allocation. State, county, and municipal health authorities can determine procedures depending upon the special needs of their communities and the usual established channels of distribution from the manufacturers.

Improper allocation and slow distribution could easily void the great efforts spent in surveillance, strain identification, and vaccine standardization and production.

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PUBLIC ACCEPTANCE OF INFLUENZA VACCINATION PROGRAMS[1]

IRWIN M. ROSENSTOCK[1]

Introduction

Background

A vast literature has been developed on the epidemiology of the Asian influenza outbreaks of 1957. There have been many reports on the development and trial of vaccines; equally well documented are plans for prevention or control of the epidemic. On the other hand, surprisingly little information is available on the public's response to the threat of epidemic or on the reasons for their response.

The present paper will report selected findings drawn from a Public Health Service study of the impact of Asian influenza upon community life in five American cities (1).

The thesis of this paper will be that public concern with influenza in 1957 was apparently not great and that fewer people were vaccinated than was previously thought. It will further be argued that the lack of concern with influenza was not confined to the general public, but was also observed in professional groups. An attempt will be made to analyze the causes of this attitude and to draw implications for the planning of future control programs.

National Recommendations

It will be recalled that in the summer of 1957 meetings were held with many planning groups, including the American Medical Association, the Association of State and Territorial Health Officers, the American Hospital Association, the Public Health Service, and the major national voluntary agencies. These groups, agreeing that an epidemic of influenza was imminent, developed recommendations for local planning of vaccination, information, home nursing, and home-care programs. Recommendations were submitted by each organization through its usual channels to state and/or local counterparts (2).

Local Response to the Threat of Influenza

Community Response

The studies made in five cities showed that policies on vaccination varied widely. In one city systematic efforts were made to control vaccine supplies and distribution and to inoculate on a priority basis, while another city developed no community program whatever. The other three cities developed limited programs. With respect to other national recommendations, attempts were made in three cities to keep the public informed about the local situation, but efforts were inconsistent or lacking in the other two cities. While additional home nursing services were deemed necessary by official or voluntary health agencies in three cities, no preparations to enlarge them resulted. In none of the cities were plans made to develop new homemaking services or to augment existing ones.

Public Response

In two of the five cities studied (the two with the most highly developed vaccination programs), it was possible to obtain detailed data from interviews with representative samples of the general public comprising 1,600 families or approximately 4,000 individuals. In one of these cities, 23 per cent of the population were vaccinated; in the other, 13 per cent. Since at least some portion of the vaccinated were members of essential or priority groups whose vaccination was almost compulsory, it would appear that the public's voluntary use of the vaccine was not great. This was true despite the fact that health officials in both cities reported that their supplies of vaccine had increased progressively and that the need to restrict vaccine to priority groups had passed before the interviewing of the general public was undertaken.

In both cities the study showed that only 4 per cent of the families interviewed actually made any preparations for influenza other than vaccination. Such preparations might have included stocking up on staple foods and medicine,

[1] From the Behavioral Studies Section, Public Health Service, Department of Health, Education, and Welfare, Washington 25, D. C.

arranging for home and child care, and seeking training in care of the sick. In summary, data obtained from these two typical cities strongly suggest that *community* preparations to meet the impact of influenza were varied but limited. On an *individual* basis, the vast majority of families took no action to ward off the threat of the disease.

Determinants of Local Response to the Threat of Influenza

Determinants of Individual Action

In attempting to find the reasons for the apparent lack of response, let us consider the findings of other studies on why the public does or does not accept such preventive health services as polio immunization (3), topical application of sodium fluoride (4), and studies on their decisions to participate in case-finding programs for tuberculosis (5) and cervical cancer (6).[2]

Only the barest outline of the findings will be attempted here. Briefly, they concern the individual's beliefs about his vulnerability to disease, its severity, and available means for combating it. Other things being equal, a person will not take action to ward off a disease unless he believes, first that he is susceptible to it; second, that its occurrence would be a serious matter; and third, that effective and acceptable means for preventing or controlling it exist and are available to him.

What did the groups in this study believe about influenza and influenza vaccine? While most members of the general public had favorable opinions about the vaccine, the vast majority did not believe that they or members of their families would contract influenza. Not more than 25 per cent of the 1,600 families interviewed even accepted the *possibility* that someone in the family might contract the disease. Regarding beliefs about the severity of the condition, it was quite evident that concern was low. Surprisingly, perhaps, less than one-half of the general population even believed that Asian influenza was more serious than the usual common cold, grippe or "flu." Less than 3 per cent of the population believed that contracting influenza would require any marked changes in their daily activities.

Thus, most members of the general public did not believe they were vulnerable to Asian influenza, nor did they believe that contracting it would be a serious matter. This combination of beliefs made Asian influenza a matter of low concern. Consequently, despite the favorable opinion about the vaccine, little public action was taken.

Determinants of Community Action

The explanation for the kinds of action that were taken by community agencies is somewhat more complex than for individuals but follows essentially the same pattern. Public health officials and representatives of voluntary health agencies tended to believe that widespread influenza outbreaks (affecting up to 20 per cent of the population) had a reasonably high probability of occurrence in their communities; that its occurrence could have rather serious effects on community functioning, and that vaccination should be fairly effective in conferring immunity. They tended to believe that systematic programs of public information were required. Moreover, they believed that programs to provide home-care services to those who needed them would probably be required in the event of an outbreak.

Officials of medical societies tended to agree with other groups that vaccination could be at least moderately effective in conferring immunity. However, while they agreed with public health officials that up to 20 per cent of the population might be stricken with influenza, they did not believe such incidence would be significantly higher than was usual for the season. More important, *medical society officials did not believe an influenza outbreak would have any serious consequences for their communities.*

Role of the Local Medical Society

In accounting for actions taken by community agencies, it must be kept in mind that beliefs of influential decision-makers carry much greater weight than the beliefs of others. In the present study it was possible to identify the medical society in each city as the one key group that could determine what kind of action, or whether any action, would be taken. While it was to be expected that the medical group would have the central responsibility for action on influenza, it was unexpected to find that their authority was relatively complete. For example, efforts of several voluntary agency people to stimulate pro-

[2] A more general discussion of explanatory principles is provided by Hochbaum (7) and Kegeles (8).

grams were dropped when medical society officials informed them that the proposed actions were unnecessary. In other instances, the submission of a plan by a voluntary agency to the medical society ended with a "pocket veto," i.e., the medical society simply did not discuss it.

The influence of the medical society extended beyond purely medical matters. In one of the cities, certain key officials of the medical society occupied positions that permitted them to influence the release of local news pertaining to influenza. In that city, virtually no local news on influenza was released to the press and no data on school absenteeism was released until the middle of the fall. At that time, local newspapers obtained a court injunction requiring the school board to release such figures.

In contrast, when medical society officials attempted to set up a vaccination program, it was established in a highly efficient manner permitting the orderly, systematic vaccination of the key groups covered. In each of the five cities it became evident that, while ideas could emanate from a variety of sources, their sponsors had ultimately to obtain the active approval of the medical society before successful programs could be developed. Health officers, chiefs of nursing services, and executive secretaries of voluntary agencies were all aware of the fact.

It is thus clear that communities responded only partially to national recommendations because officials of the influential local medical societies tended to believe that an influenza epidemic did not pose serious problems.

If this is true, why did medical societies take any action? Why did four of the five communities establish vaccination programs, albeit varied ones? No definite answer to these questions can be given, but a few pertinent observations may be made.

In two cities, prior experience with local poliomyelitis immunization programs had resulted in mutual distrust between public health officials and the medical societies. Various circumstances had resulted in more extensive public polio vaccination programs than were deemed necessary by the local medical societies and the health officials believed that this was due to a laissez-faire attitude on the part of the societies. Consequently, when confronted with the influenza crisis, these societies took firm action to prevent a recurrence of the earlier happenings. No evidence was obtained to indicate a similar background in the three remaining cities.

Clinical and Public Health Orientation

Inferences drawn from the data suggest some basis for the different views of medical society and public health officials about the anticipated epidemic. It appears that the answer may lie in the different orientations of the two groups toward the nature of health problems. The primary concern of public health agencies was that the anticipated high prevalence of illness might seriously impair or overtax normal community services. In short, the *number* of persons who might become ill was the point of concern for public health workers.

Officials of medical societies tended to be less concerned with the *number* of individuals who might be stricken than with the *clinical seriousness* of the disease in those who might contract it. From that viewpoint, the private practitioner might regard a single case of paralytic polio among his patients as much more serious than a 50 per cent attack rate of influenza.

Medical society representatives tended to look at the disease in terms of the impact on the individual patient, while voluntary and official health agencies tended to look at the disease in terms of the impact on the community. For public health groups, then, the orientation was to the *community as patient*; for medical societies the orientation was to the *individual as patient*.

National Recommendations and Local Orientations

Much of our nationally derived information served both orientations. For example, the Surgeon General of the Public Health Service in addressing an earlier conference of state and territorial health officers, had said:

> From the point of view of the individual, we feel there is little cause for special anxiety or undue concern ... the 10 to 20 per cent of the people who might contract Asian influenza would have a relatively mild illness .... From the point of view of the medical profession and the public health specialist, however, the problem takes on a somewhat different aspect and has special interest because of its potential effects on a community as a whole. (9)

In general, the single statement that the outbreak was likely to be highly prevalent but not clinically serious could be interpreted by public

health workers as *constituting a threat to the community* and by clinicians as *not constituting a threat to the individual.*

Conclusions

Four principal conclusions may be drawn from the foregoing material:

(*1*) Policies and programs agreed on and transmitted by national groups were not consistently reflected in local action. In the American communities studied, vaccination programs and other measures for controlling influenza did not achieve the level of individual and community acceptance desired by national groups.

(*2*) Whether *individuals* would take action to prevent influenza depended mainly on three factors: their beliefs in their vulnerability to the disease; their beliefs about its seriousness; and their beliefs about the efficacy of preventive measures.

(*3*) Whether *communities* would take action depended largely on the relevant beliefs of those in authority to make health decisions. In the five cities studied the local medical society was crucial in determining the kind of action that would be taken relative to influenza.

(*4*) The different orientations to health problems of clinicians and public health workers may provide a basis for understanding how communities behaved in response to the influenza threat in 1957.

Although the findings summarized here, as well as others not included, have important implications for the planning and operation of public health programs, time will not be taken now to spell them out. It may be suggested, however, that effective disease control depends not alone upon knowledge of the epidemiology of disease. It depends as much upon knowledge of the *epidemiology of community decision-making and action.*

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ASIAN INFLUENZA VACCINATION: DOSAGE, ROUTES, SCHEDULES OF INOCULATION, AND REACTIONS[1]

GORDON N. MEIKLEJOHN

With almost three years of experience with the Asian strains of influenza now behind us, it is interesting to look back on many of the problems which were met in 1957 and to interpret them in the light of information which has developed since that time. From the standpoint of vaccination, the situation which existed during 1957 was unique in that essentially the whole population lacked experience with the dominant antigens of the Asian strain and, for this reason, reacted quite differently and far less effectively than with earlier strains. The striking antibody response, which had been evoked with relative ease with A1 or A antigens during earlier studies, was notably lacking in persons who received the early Asian vaccines.

The present situation is very different as a result of further seeding of the population with this virus. In the Denver area, for example, since the major epidemic during the fall of 1957, we have experienced three additional A2 outbreaks, the most recent occurring in early 1960. As a result, the great majority of the persons with whom we are dealing now have A2 antibody, and the situation has come to resemble that which existed during the latter part of the A1 era. The elevation of antibody titers by vaccination has become a far simpler task since a booster effect is now obtained in persons who have already had prior experience with the A2 virus. Post-vaccination antibody titers are far higher than the convalescent antibody titers of persons who were ill with A2 influenza in 1957. This change in the situation is pertinent in the discussion which follows, for the factors which affect the usefulness of vaccination are quite different in 1960 from those which existed in 1957.

Dosage

In 1957, single injections of A2 vaccines of 200 chicken erythrocyte agglutination (CCA) unit potency were only moderately effective in elevating hemagglutination-inhibition antibody titers, and a very substantial number of persons showed no measureable increase in titer. With vaccine of 400 CCA unit potency, the response was somewhat better. It thus appeared that, when a single injection of vaccine was given to a "susceptible" population, increasing potency produced greater increase in antibody titer, at least up to this point. This situation presumably still holds for persons who continue to show no A2 antibody.

During the fall of 1958 we had occasion to test specially standardized A2 vaccine which contained 100 CCA units. This material was given to groups of Air Force personnel in amounts of 1.0, 0.5, and 0.25 ml., representing 100, 50, or 25 CCA units. At the same time, we had given a commercial vaccine containing 400 CCA units to another group of men. From the results of hemagglutination-inhibition antibody tests with serum from these men, it is clear that the antibody response improved as potency increased from 25 to 100 units and that there was a further rise in persons who received the 400 CCA unit material. A comment should be made here that we are dealing in this study with two different populations rather than one, namely, one without antibody and another which had already received its basic immunizing dose. The results clearly reflect this difference.

At the present time, it seems desirable to provide 400 CCA units of A2 strain in polyvalent vaccines. In the not too distant future, this relatively large amount of antigen may be unnecessary, since smaller amounts appear to be highly effective in elevating the antibody titer of persons who already have antibodies.

With adjuvant vaccines the situation is somewhat different. These also proved disappointing in evoking antibody in persons who had no preexisting antibody. However, as with earlier strains, they showed a striking booster effect in those who already possessed antibody in measureable amounts. One hundred CCA units of Asian antibody prepared with adjuvant of the type developed by Salk, namely, mineral oil and arlacel, produced elevations in antibody comparable to those seen with aqueous vaccine containing 400 CCA units.

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Route of Administration

Considerable interest has been focused on the relative effectiveness of vaccination by the intradermal compared with the subcutaneous route. It is difficult to present a clean interpretation of the rather voluminous studies which have been reported. It would appear, however, that, when small doses of antigen are used, the response following intradermal injection may be comparable to, or better than, that observed when 1 ml. of the same vaccine is given by the subcutaneous route. However, as the amount of antigen in the vaccine is increased to the levels suggested above, the antibody response following administration of 1 ml. subcutaneously is uniformly considerably greater than that seen following intradermal injection of 0.1 ml. of the same material. For this reason, in the adult the subcutaneous injection of 1 ml. of vaccine appears preferable from the standpoint of evoking greater antibody response. The intradermal route still holds a place of usefulness in the vaccination of children, in whom serious systemic reactions have been reported with the use of larger amounts of subcutaneously administered vaccine, and in those adults in whom it is undesirable to produce systemic reactions. Jet injection holds promise but still has not received wide application.

Before leaving the topic of route of administration, a few comments on live intranasal vaccine might be of interest. We have, during the past year, given Russian vaccine to 27 subjects and crude allantoic fluid from Russian seed virus to an additional 9 subjects. Time, I fear, does not permit proper acknowledgments to the many persons who made this material available. The results we obtained raised a number of questions, and I hope Dr. Soloviev will provide some of the answers in the next presentation.

With inactivated, injected vaccines, most persons without antibody showed increases in titer and, in addition, people with antibody usually showed a sharp booster effect even though their initial titer might have been quite high.

With the live vaccine the response of persons without antibody was similar, but significant rises in titer were not observed in most persons who had antibody at the time of vaccination. Only 2 of 20 persons showed a fourfold increase in titer. For this reason the post-vaccination antibody titers of the group were considerably lower than those observed with current inactivated vaccines. This could well be important as A2 variants appear, for it is not unlikely that those persons with very high Jap-305 antibody titers would have enough heterologous antibody to provide protection against new variants, while those with low antibody titers would not.

Schedules of Immunization

It was demonstrated in early studies of influenza virus that a single injection of vaccine evoked a relatively satisfactory antibody response and that a second injection of the same material produced little additional increase in antibody titer. Single injections of vaccine given in the fall of each year were consequently recommended.

With the appearance of the A2 strains, it soon became clear that the same number of CCA units of vaccine, when divided into two injections, produced a response better than was obtained when given in a single injection. The optimal interval for obtaining maximal effect is still not clearly defined, but it would appear to be one of more than four weeks between injections. While a double schedule of immunization unquestionably was desirable in 1957 and possibly in 1958, the need for a second injection is diminishing as A2 epidemics continue to go through the population. At the present time, in the group which we are studying, 84 per cent had measurable A2 antibody titers prior to the administration of vaccine. The 16 per cent who lacked antibody would unquestionably have benefited from the double immunization schedule. However, from the practical standpoint, it is dubious that this will be justifiable as the "susceptible" group diminishes further in size.

Dr. Mogabgab at Tulane University and our group restudied this matter at length last winter and again found relatively little evidence of booster effect with second injections. We have compared the response following first and second injections of a polyvalent aqueous vaccine containing A2 and A1 strains. With the A2 strains a modest post-vaccination increase of antibody titers is observed after the second injection. With the A1 antigen—and this held for other influenza A and B strains also—very little change in titer was noted following the second injection.

It has been customary to recommend annual vaccination because of the antibody titer drop

during the year following vaccination. There is very little direct evidence on the need for such a program. We have obtained a very limited amount of data from a study of Air Force personnel who have been vaccinated five times during the past four years. Granted that the numbers are small, little support is added to the thesis that annual vaccination serves a useful purpose. This remains an area which should be further explored.

The duration of immunity following adjuvant vaccines remains to be determined. Salk's earlier studies indicated that antibody persisted considerably longer than following administration of aqueous vaccines, but further work is needed before it can be said whether this persists for two, three, or more years. The post-vaccination antibody titers obtained with vaccines given in the fall of 1959 illustrate the magnitude of the antibody increases which can now be obtained.

Some of you may have been disturbed by the inference that antibody levels and protection are one and the same. I would support this thesis only to this extent, namely, that cases of influenza occur with negligible frequency in persons with high homologous antibody levels. The persons vaccinated in the fall of 1959 fared very well during our recent A2 epidemic. Here the degree of protection provided by the adjuvant vaccine was comparable to, or better than, that observed in the past with aqueous vaccines.

Reactions

With aqueous vaccine two types of reaction have caused difficulty in the past. The first of these may be described as toxic or systemic and is manifested by fever, chilliness, myalgia, headache, and lassitude appearing between six and eighteen hours after vaccination and usually disappearing within twenty-four hours. These have been reported to be more common with the higher potency preparations in use during recent years, and rates as high as 15 per cent or more have been reported. While these have generally been attributed to the amount of virus antigen in the vaccine, there is evidence that impure preparations tend to have produced a higher reaction rate than highly purified ones, and the relative importance of the two components is still not entirely clear. In the adult, reactions of this sort are ordinarily not severe enough to cause incapacity. In military groups, such as the ones with which we work, it is rare to have more than two or three men per thousand admitted to the hospital following vaccination with material of 1,000 CCA unit potency.

Allergic reactions have also been reported in varying frequency. The true incidence of these is difficult to assess since most persons who have reported on influenza vaccines have carefully excluded from their study any individual with recognized allergy to chicken or egg. Anaphylaxis has been reported very infrequently, asthma somewhat more often, and a variety of rashes ranging from macular to urticarial have also been observed.

With adjuvant vaccines, a special type of local reaction deserves comment. If these materials are injected subcutaneously, cysts, which persist for many months, develop in a certain number of the recipients. These may occur following intramuscular deposition of vaccine, but certainly at a far lower rate. While these were reported to be frequent in earlier studies, they have rarely been observed with the preparations tested by the Influenza Commission during recent years. As Bell pointed out in his earlier studies, these may pose a special problem in children and in people with pigmented skins. Sensitization to egg proteins has been a source of some concern, but to date no harmful responses of this nature have come to light.

In summary, the following conclusions may be drawn:

Antibody for A2 strains may now be produced by vaccination as readily as with strains which were prevalent during the period from 1940 to 1957. Both aqueous and adjuvant vaccines produce highly satisfactory response, but the amount of antigen needed for the latter is less and the period over which antibody titers remain elevated is longer. A double schedule of injections of vaccine serves little purpose with the older influenza strains and its value in raising A2 antibody-titer response diminishes as the immune status of the population increases. The frequency with which vaccination should be repeated needs further study.

SOME RESULTS OF THE STUDY OF ASIAN INFLUENZA[1]

V. M. ZHDANOV AND V. D. SOLOVIEV

EPIDEMIOLOGIC CHARACTERISTICS

The 1957 pandemic of influenza broke out in some countries in the form of one wave; and in other countries, in the form of two waves. The first wave occurred in the spring-summer season; and the second, a maximal wave in the autumn. The first serious outbreaks of the disease in the USSR were observed in the territory of the Central Asian Republics, especially in Usbekistan. Tashkent, the capital of this Republic, had its most intensive outbreak in May. The incidence of the disease increased ninefold in May as compared with the incidence in the preceding month. In terms of time, however, this spread of Asian influenza was specific only to certain southeastern parts of our country. There was but a comparatively small increase in the incidence of the disease in the rest of the Soviet Union during the summer months. This small increase, however, led to a wide spread of the infection.

The second wave of the pandemic developed in late September and flourished in October, when there occurred the maximal increase in the incidence of the disease, the outbreak of which was observed almost simultaneously in a majority of the cities. At the same time, a notable, but considerably lower, increase in the incidence of the disease was observed in Tashkent. The same picture was also noted in other regions where the first wave of the pandemic was the most intense. The incidence of influenza gradually decreased in November and later in the year, but throughout the year it was at a higher level than normal for previous years.

After a lapse of thirteen to fourteen months there was again a general increase in the incidence of the disease in January and February, 1959, which quantitatively was lower than 1957 levels. On the other hand, in some cities it was higher than during the pandemic of 1957.

If judged by the principal characteristics, the outbreak of 1959 was not essentially different from pandemic influenza. The highest incidence of disease occurred among school children and infants. In the majority of cases the clinical course of the disease was either mild or moderate. The highest mortality was noted among small children and older people.

Laboratory investigations demonstrated that the strains of virus A2, isolated in 1959, corresponded in their antigenic structure to the strains of virus A2, isolated in 1957. However, one circumstance deserves special consideration. Among those with the disease there were people who had had influenza in 1957. This group comprised 20 to 35 per cent in Moscow and up to 51.4 per cent in Kiev.

The incidence of influenza and other influenza-like illnesses (rate per 10,000 population per month) are shown in figures 1 to 5 for the years 1957, 1958, and 1959. To provide representative examples, the cities chosen are located in different parts of the Soviet Union and give an idea of the types of outbreak and their spread in time. Evidently, unlike the previous years, the maximal incidence of the disease in 1959 occurred during 2 to 2.5 months. This prolongation can be accounted for by the fact that, in addition to virus A2, virus B was also involved in the epidemic process. In other words, the epidemic of influenza in 1959 was of mixed etiology.

We do not have definite evidence which would allow us to reconsider the common view that in cases of influenza man alone is the source of infection. At the same time, it should be pointed out that, relevant to virus A2 serologic investigations of the blood of 100 cows and 60 pigs conducted in the summer of 1957 yielded negative results. However, in November of the same year, newly conducted research led to the detection of antibodies which definitely neutralized virus A2 in the sera of 20 of 60 pigs. Influenza antibodies were not detected in the sera of the cows. The investigations of the blood of animals were conducted at the suggestion of M. M. Kaplan and A. M.-M. Payne of the World Health Organization, Geneva.

The observations should be taken into account that were carried out by M. G. Gaidamaka (1958), who reported on an outbreak of influenza-like diseases among horses in Kharkov and

[1] From the USSR Regional Influenza Centre, World Health Organization, Geneva, Switzerland; and the Influenza Committee of the Ministry of Health of the USSR, Academy of Medical Sciences, USSR.

who also stated that he detected antibodies against virus A2 in the blood of horses who were previously ill. Here, also, the observations of Z. V. Domracheva should be mentioned who reported in January, 1959, on the outbreak of the disease among horses at the Moscow hippodrome. In terms of time these influenza-like diseases coincided with the influenza epidemic among human beings. The strains of the virus isolated from the throat washings of horses showed no difference from the strains isolated from the workers of the hippodrome who had influenza which proved to be similar to virus A2. There was an increase of specific antibodies in the blood of the horses, both for their homologous strains and the strains of virus A2. Additional data are required in order to pass a final judgment on the nature of these diseases. At the present time it can only be assumed that the horses were infected with the human strain of the influenza virus.

Concerning the Problem of the Clinical Picture and Pathogenesis of Asian Influenza

Clinical observations made it possible to state that in the summer of 1957 during the first wave of the pandemic, with outbreaks of influenza in some parts of the country, the main symptoms of the disease were fever and general prostration. In the majority of cases the temperature rose to 38°C. or even higher, headaches developed, as did pains in the back and muscles, accompanied by a feeling of general fatigue and lethargy. The characteristic peculiarity in the clinical course of influenza in May and June, 1957, was the development of mild inflammatory phenomena in the upper respiratory tract, together with the appearance of symptoms unusual in cases of influenza: nausea, vomiting, and diarrhea. We are inclined to consider these last symptoms as manifestations of general intoxication. We frequently observed changes in the reticuloendothelial system and enlargement of the spleen, liver, and especially lymph nodes.

The second or autumn wave of influenza was characterized by a more complicated clinical course. Apart from general intoxication, the majority of patients developed inflammation of the upper respiratory tract which sometimes caused complications, the most common being pneumonia. The complications were not only secondary infections (mostly staphylococcal), but were also connected with the spreading of the virus in the lungs.

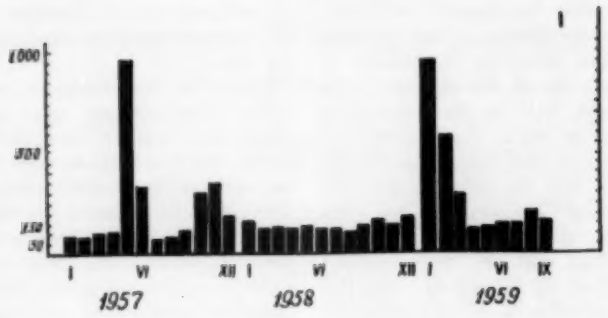
During this time increased periods of absenteeism, which averaged up to seven days, were noted; in comparison, the epidemics of influenza A, A1, and B of previous years caused an average period of absenteeism not exceeding five days. The disease developed in two phases rather frequently. The cases substantiated by isolation of the virus demonstrated that, when the disease took the two-phase course, the length of the remission was from two to five days. During the second phase, which, as a rule, took a more complicated course than the first one, virus A2 was isolated on the seventh to twelfth days of the disease.

The asymptomatic forms of influenza occurred more seldom and comprised only 8 per cent of the cases among adults, while in previous years up to 50 per cent of all cases were asymptomatic.

The pathologic investigation of fatalities following influenza showed that the main cause of death was pneumonia (up to 95 per cent). The autopsies demonstrated focal bronchopneumonia as well as hemorrhagic diffuse pneumonia. According to the studies of A. P. Avtsin, hemorrhagic foci were detected in the visceral organs, including the cardiac muscle; edema of the brain also developed. In early deaths, phenomena of general intoxication prevailed, sometimes without lesion of the respiratory organs. Thus, the pathologic findings, too, indicated generalized involvement of the body regions with which symptoms had been associated in the clinical manifestations of the 1957 influenza.

The clinical picture of influenza A2 in 1959 exhibited the peculiarities of the 1957 Asian influenza. In 1959 there were more complicated forms of the disease, but other symptoms were the same, i.e., general intoxication prevailed over the local respiratory disorders.

In the work of earlier Russian physicians, influenza has been considered to be a generalized disease, with the general intoxication of the host playing a very important role along with the local inflammatory processes. The local effect of the causative organism of influenza is necrosis of the epithelium and inflammation of the mucous membrane of the respiratory passages in mild cases, the necrotic hemorrhagic pneumonia in severe cases. The general toxic effect of the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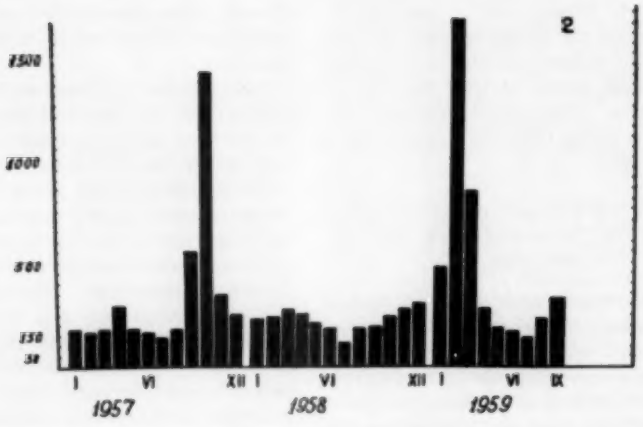
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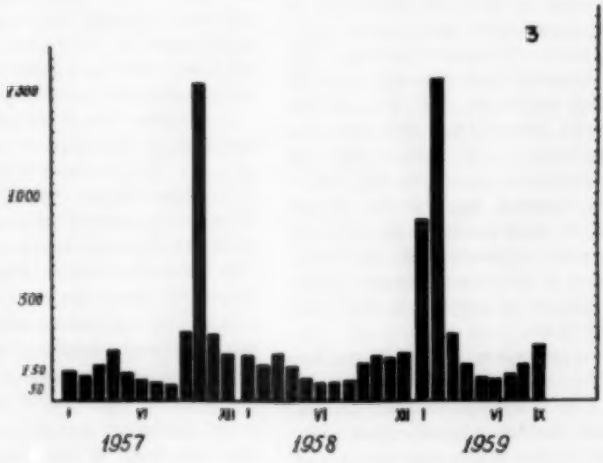
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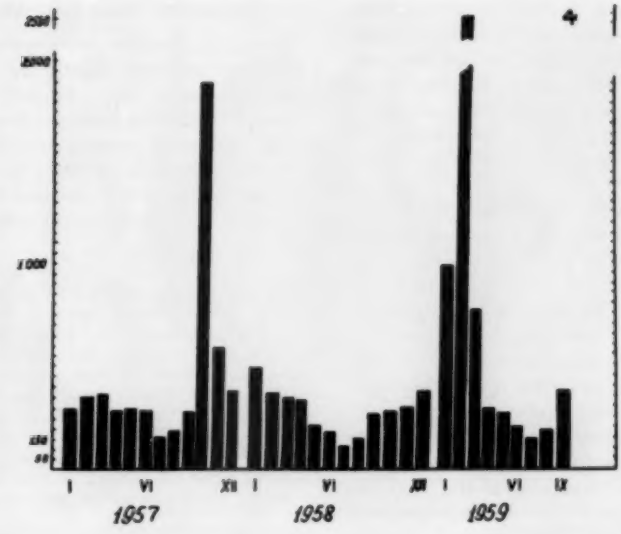
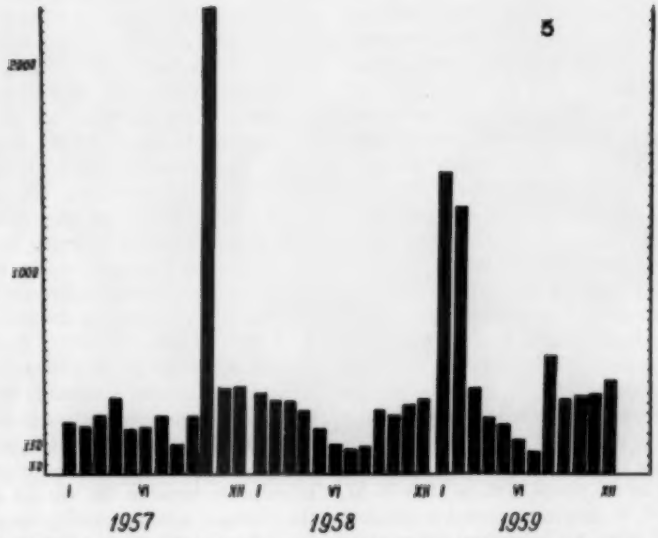




FIGS. 1–5. The incidence of influenza and other influenza-like illnesses (rate per 10,000 population per month) for 1957, 1958, and 1959.

Fig. 1. In Tashkent
Fig. 2. In Minsk
Fig. 3. In Kiev
Fig. 4. In Baku
Fig. 5. In Moscow

causative organism of influenza is mainly manifest in lesions of the nervous system and cardiovascular system, and in the inhibition of the host defenses.

On the basis of the latest experimental data and clinical observations, the pathogenesis of influenza can be described as follows: As soon as the virus touches the mucous membrane of the

respiratory passages, it starts a fermentative action with the mucoid substance of the secretions of the respiratory passages and the membranes of the epithelial cells. It is only through contact with the epithelial cells (the membranes being no longer intact) that the penetration into the multiplication of the virus within the cells is at all possible.

This process develops in cycles. The cells are destroyed, together with the intracellular matter rich in mucoids, while the virus is multiplying. The necrosis of the cells and the disintegration of the intermediate matter leads to the development of a reactive inflammatory process. The permeability of the tissues is increased and immunologic ability is weakened, due to the inhibition of the phagocytic activity of the leukocytes.

While the process develops further, new generations of the virus from the cells are absorbed on the walls of the capillaries of the lungs, the capillaries are destroyed, and hemorrhagic pneumonia ensues. The mass penetration of the virus into the blood is manifest in the functional activity of the nervous system, and leads to an increase in the number of vascular disorders, especially capillary circulation, not only in the lungs but in other organs as well.

In severe cases death occurs, due to hemorrhagic pneumonia or lesions of the vital organs, or to secondary infection in long-term cases. However, these outcomes are rather rare. Usually the host overpowers the infection very quickly, and the multiplication of the virus is checked. The virus which penetrates into the blood is carried through the kidneys into the urine. The bacteriologic microflora which penetrated into the tissues is destroyed; a process of repair develops, and specific immunity increases. Investigation carried out at the time of the Asian influenza pandemic confirmed the presence of the virus in the blood of patients and its penetration into the urine.

In addition to the above data, in 1959 V. M. Zhdanov and V. V. Ritova succeeded in isolating two strains of virus A2 from the cerebrospinal fluid of patients suffering from the meningoencephalitic syndrome. These findings are of interest, since the number of cases of cerebral complications is on the increase. The complications are connected etiologically with influenza and probably result from penetration of the virus into the central nervous system.

Laboratory Diagnostic Methods

Among the methods of laboratory diagnosis which were employed during the pandemic of Asian influenza in 1959, the method of rhinocytoscopy yielded the most positive results. This clinical laboratory method was developed by E. A. Kolyaditskaya in 1947; in essence it is the investigation of cells obtained from the mucous membrane of the upper respiratory passages.

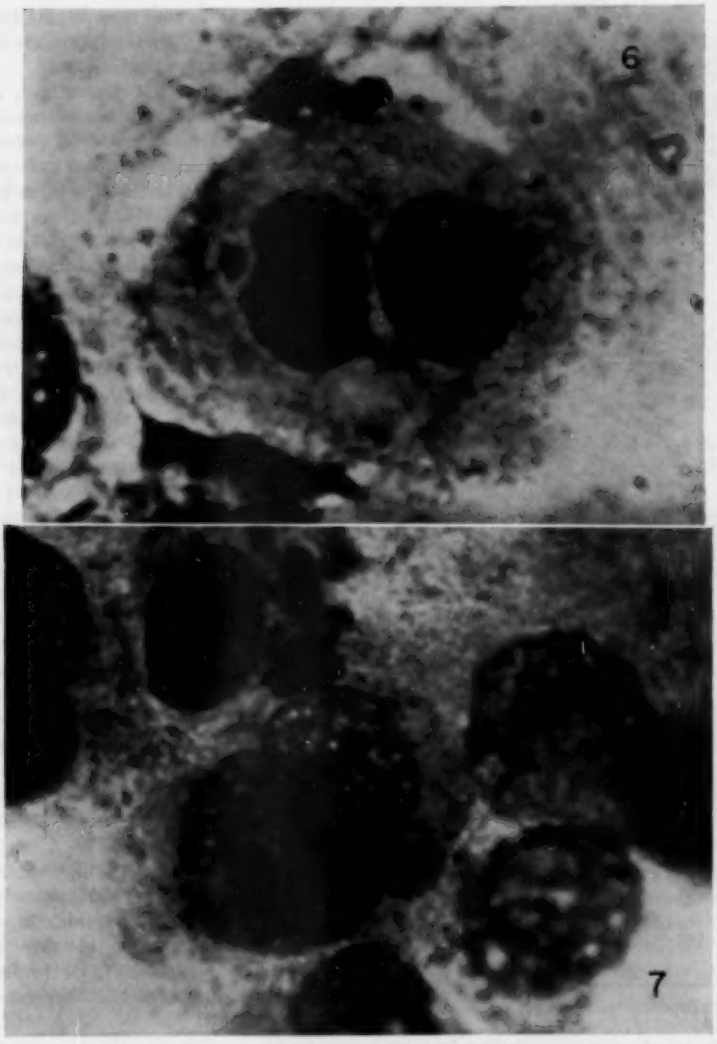
In order to obtain the material, a narrow polished glass or plastic plate is placed in the lower nasal cavity of the patient. The cells of the cylindrical epithelium stick to the plate; these cells, isolated from patients with influenza, exhibit characteristic features of the disease. After fixing and staining the material, microscopic study is made to determine the nature of the observed cellular elements (figures 6 and 7). The diagnosis of influenza is established when the layers of the cylindrical epithelium are observed under the microscope.

In addition to the layers of epithelium, there may be many epithelial cells, the number of which is recorded by means of crosses (*see* table 1). (Four crosses denote the maximal number of cells within the field of vision.)

Rhinocytoscopy yields the best results when it is used within the first two to three days of the disease. Recently, V. E. Pigarevsky and others have detected specific inclusions in the affected cells. As is evident from the reports of various investigators, the correspondence of the clinical and virologic diagnosis to the data of rhinocytoscopy fluctuates within the margin of 60–80 per cent. Intracellular inclusions are not as frequent. According to the data obtained by A. I. Slobodyanuk in 1959, the cells of the cylindrical epithelium in patients suffering from Asian influenza were detected in 63.4 per cent of cases and intracellular inclusions in 32 per cent.

Observations carried out at the clinic of the Institute of Virology demonstrated a regular relationship between the clinical diagnosis and the virologic, serologic, and cytoscopic investigations. In table 1, the comparative data obtained from studies pertaining to the 1959 influenza outbreak are shown.

As is evident from table 1, there is a correlation between the results of rhinocytoscopy and other methods of detecting influenza. Rhinocytoscopy gives the best proof when one can observe the layers of the cells of the cylindrical epithelium



FIGS. 6 and 7. Cylindrical nasal epithelium and specific inclusions under the microscope. (× 2000)

under the microscope. Nevertheless, in control investigations it was found that similar findings may occur with certain other conditions (angina, tonsillitis, pharyngitis).

ISOLATION OF VIRUS FROM PATIENTS

The best method of isolating virus A2 from patients is the introduction of their throat washings into the amniotic cavity of the ten-day chick embryos. Our experience proves that the employment of monolayer cultures of the epithelial cells of man and monkeys with the hemadsorption indicates that the virus is a valuable supplement to the existing methods. This broadens the investigative possibilities.

At the time of the 1957 pandemic, the strains

TABLE 1

EXAMPLES OF CLINICO-LABORATORY DIAGNOSES OF INFLUENZA

Number of Patients	Virus Isolated	Leukocytic Reaction of the Blood	Rhinocytoscopy		Coefficient of the increase of Antibodies	Comment
			Cylindrical Epithelium	Presence of Inclusions		
1	A2	LP*	+†	−‡	2	
2	A2	LP	−	−	2	
3	A2	LP	+++	−	2	
4	A2	LP	+++	−	2	
5	A2	LP	++++	+	2	
6	A2	LP	++++	+	2	
7	A2	LP	+++	−	2	
8	A2	LP	++++	−	2	
9	A2	LP	++++	−	2	Pneumonia
10	A2	N§	+	−	2	
11	A2	N	++	+	4	
12	A2	N	++++	+	4	
13	A2	N	+	−	4	
14	A2	LP	+++	−	4	
15	A2	LP	+++	−	8	
16	A2	LC‖	+++	−	8	Pregnancy
17	A2	N	++++	−	8	
18	A2	LC	+++	−	8	Pregnancy
19	A2	N	+++	−	16	Pneumonia
20	A2	LP	++++	−	16	
21	A2	LP	+++	−	16	
22	A2	LP	+++	−	32	Pneumonia
23	A2	LP	+++	+	8	
24	−	LP	++++	+	2	
25	−	LP	−	−	4	
26	−	LP	−	−	4	
27	−	N	−	−	16	
28	−	LP	++	−	0	
29	−	LP	++++	+	0	
30	−	N	++++	−	0	
31	−	LC	+++	−	0	Folicular angina
32	−	LC	++	−	0	Folicular angina

* LP—leukopenia

† +, ++, +++, ++++ denote number of cells; ++++ indicate innumerable cells within the field of vision.

‡ — negative result

§ N—normal quantity of leukocytes

‖ LC—leukocytosis

of the virus isolated from patients were homogeneous in their antigenic structure and did not differ essentially from the strains isolated in Singapore, Japan, and the United States. The differences between the strains were manifest chiefly in a lesser or greater sensitivity to normal inhibitors (avid and nonavid strains) and in their capacity for the agglutination of erythrocytes of different animals.

The isolation of the strains from patients in 1958–1959, according to the information of the Influenza Centre of the USSR, yielded the following results. Of 394 strains isolated in 1958, 337 were virus A2 and 6 were virus B. In January, February, and March, 1959, 756 of 1,081 isolated strains were virus A2 and 64 were virus B. The work of identifying other strains is not yet complete. According to the data obtained by A. S. Gorbunova in studying strains of virus A2 isolated in 1959, there was no well-pronounced division into avid and non-avid forms with respect to normal inhibitors. Rather, they corresponded to the P-R variants of Mulder. At the same time, Gorbunova detected some specific peculiarities in the freshly isolated strains of virus A2 and of previously isolated viruses. These peculiarities were established by means of studying antisera.

In table 2 the cross-reaction in the hemagglutination inhibition reaction is illustrated; this shows the maximal titers of anti-hemagglutinins to the Singapore strain of virus A2. On the basis of this difference, the strains are subdivided into Singapore-positive and Singapore-negative ones. The significance of this deviation is not clear, but in all probability it indicates that variations of the influenza A virus are continuing.

Of interest is the antigenic structure of strains of virus B isolated in 1959. In table 3 are summarized the investigations of A. S. Gorbunova which allow us to draw the conclusion that variations in the strains of influenza virus group B are also continuing. We think it advisable to divide the virus B group into three subgroups: B (prototype, Lee strain), B1 (prototype, Bon and Kri strains), and B2 (prototype, 1959 strains).

TABLE 2

IDENTIFICATION OF STRAIN A2 WITH IMMUNE SERA OF WHITE RATS (HEMAGGLUTINATION-INHIBITION TEST)

Virus	Immune Sera	
	A2/Singapore/1957	A2/Cheljabinsk/1959
A2/Cheljabinsk/1959	1,280	320
A2/Singapore/1957	160	0

Specific Prophylaxis of Influenza with Intranasally Administered Live Vaccine

During the past twenty years, research has been under way in the Soviet Union aimed at the elaboration of the technology of production of a live vaccine. This research has also been concerned with the determination of the immunologic efficiency of the vaccine. The work started on a systematic basis in 1954 when special attention was paid to the selection of the best vaccine strains and to the production of a dried vaccine which could maintain its properties after prolonged storage. It has been found that such a vaccine must contain sufficient amounts of active virus to have satisfactory immunologic properties; the virus must be fixed easily to the mucous membrane of the upper respiratory tract of man. In meeting the minimal requirements for such vaccines, the vaccinal strain is considered suitable only when, after intranasal administration to nonimmunized persons, it can be reisolated in not less than 80 per cent of the vaccinated persons forty-eight hours thereafter.

If the vaccine contains strains which correspond to these requirements, the majority of the vaccinated persons react by developing leukopenia, accompanied by a steady increase of antibodies. In cases of intranasal immunization, the increase of antibodies is most apparent when the antibodies are determined in the nasal secretions, while they are less pronounced in the blood serum.

Three methods are recommended to ensure that the vaccine strains possess the necessary properties: (*1*) passage through the upper respiratory tract in vaccinated persons (A. A. Smorodintsev); (*2*) passages through human embryo lung tissue culture; and (*3*) the use of freshly isolated virus limited to four passages in chick embryos (A. A. Zhdanov, V. D. Soloviev).

It has been demonstrated that live vaccine decreases the incidence of the disease from 2.5- to 5-fold among vaccinated persons, provided that the type of antigen and the virus which caused the outbreak are similar. As a rule, the vaccine is quite safe for adults and does not cause any harmful reactions. However, when administered to children up to ten years of age, it frequently causes reactions clinically similar to mild influenza. The vaccine prepared from the strains of virus A2 isolated in the USSR after four passages in chick embryos was employed in the prophylaxis of Asian influenza.

TABLE 3

Antigenic Structure of Influenza B Virus Strains Isolated in 1959

Antiserum	Strains of Influenza B Virus										
	1940	1949	1952	1955	Mih	106	60	15	Mih	295	114
Lee/40	1*	1/8†	0‡	0	0	0	0	0	0	0	0
Kri/49	1/8	1	1/8	1/16	1/8	1/8	0	0	0	0	0
BM8/55	0	1/8	1	1/2	1/4	1/4	1/8	1/8	1/8	1/8	1/16
B-55/55	0	1/16	1/4	1	1/4	1/4	1/8	1/8	1/8	1/8	1/16
B-Moscow-59	0	1/16	1/4	1/4	1	1/2	1/2	1/4	1/4	1/2	1/8

* 1—homologous titer of the serum.

† Fractions are indices of antigenic relationships.

‡ 0—absence of relationships.

In the summer of 1957, V. V. Ritova, S. L. Leonidova, and S. S. Unanov carried out observations on 3,481 vaccinated persons. A general reaction to vaccination in the form of fever with temperatures up to 38°C. was observed in 5.5–7.6 per cent of persons to whom vaccine was administered in a dose of 0.3 ml.; in 4.4 per cent of those who received a dose of 0.1 ml.; and in 2.3 per cent of those who received a dose of 0.01 ml. Local reactions were not very clearly manifest and were observed principally among the persons who received the 0.3 ml. dose, which was the maximal dose used. Taking these facts into consideration along with the immunologic response, it has been decided to adopt a dose of 0.1 ml. as that which can be recommended for mass vaccination. It should be pointed out that production laboratories obtain not less than fifty doses of vaccine from one ten-day-old chick embryo.

In 1957, about 20 million doses of the vaccine were administered. However, it was too late in the pandemic to estimate its epidemiologic efficiency except among some of the vaccinated persons. The data obtained are summarized in table 4. Later, in 1958, the possibility of an influenza B epidemic arose because of the period of time which had elapsed from the last influenza B epidemic in the Soviet Union (four years) and the lack of antibodies to the strains of virus B in the blood sera of healthy people. For that reason, in 1958 vaccination was performed with two monovalent vaccines; at the beginning, vaccine

A2 was used; fourteen to twenty days later, vaccine B was administered.

Thus far we have been able to study the effectiveness of the vaccines in only a portion of those vaccinated (table 5).

Our practical experience justified our expectations: in the winter of 1959 the epidemic was of mixed etiology, which included diseases connected with viruses A2 and B, although the majority of cases were of the A2 type of influenza, as confirmed by virologic findings.

If the efficiency figures of the vaccinations done in 1957 and 1959 are compared, the conclusion can be drawn that, generally speaking, both vaccination efforts confirm the data obtained previously. This practical experience allows us to assay the method of intranasal immunization and conclude that it is promising but not yet fully satisfactory. Further research is necessary to improve the quality of the vaccine which is used for mass-specific prophylaxis. The method of immunization could also be improved.

There is hardly need to prove the ease and simplicity of intranasal administration of the vaccine in comparison with subcutaneous injections, the method of administering inactivated influenza vaccine. Experience shows that only the intranasal route of vaccination can provide for the mass immunization of millions of people within a short period of time and at minimal cost.

However, we do not consider the problem of intranasal administration of the vaccine solved. Two suggestions have been made: immunization with dried vaccine which has been diluted, and immunization with dry vaccine in the form of a powder. At the present time it is necessary to solve the problem of the most advantageous procedure for mass use which will enable the introduction of the exact dose of vaccine into the upper respiratory tract.

TABLE 4

EFFICIENCY OF INFLUENZA A2 VACCINE IN 1957

Place of Vaccination	Number Vaccinated	Morbidity	Per Cent	Number Not Vaccinated	Morbidity	Per Cent	Protection Ratio
Orekhovo-Zuevo	3,076	381	12.4	3,155	1,386	43.9	3.6
Minsk	9,310	355	3.8	17,667	2,292	12.9	3.4
Krasnodar	4,200	563	13.4	2,536	1,060	41.8	3.2
Kharkov	41,800	4,218	10.1	48,135	8,080	16.8	1.7
Leningrad	25,867	1,316	5.0	46,205	3,176	6.8	1.3

TABLE 5

EFFICIENCY OF INFLUENZA A2 AND B VACCINES IN 1959

Place of Vaccination	Number Vaccinated	Morbidity	Per Cent	Number Not Vaccinated	Morbidity	Per Cent	Protection Ratio
Moscow	900	77	8.6	1,000	343	34.3	3.9
Tbilisi	28,909	3,035	10.5	30,318	10,460	34.5	3.2
Krasnodar	9,824	1,334	13.5	5,474	2,844	52.8	3.9
Kishenev	3,449	1,035	30.4	2,102	937	44.5	1.5
Tallin	10,950	1,315	12.0	5,170	940	18.2	1.4

CONCERNING SEROTHERAPY AND SEROPROPHYLAXIS

The use of serum from immune horses introduced into the body through the respiratory tract for the treatment and prophylaxis of influenza was suggested by A. A. Smorodintsev in 1936. In recent years numerous observations established the fact that the serum, provided it contains the necessary amount of specific antibodies in adequate titer, shortens the period of the disease by 1–2.5 days and reduces the possibility of complications. Experience with the serotherapy of Asian influenza confirms previous observations. It shows the advisability of using not only the respiratory, but also the intramuscular, route of introducing the serum; immune gamma globulin or the serum of human convalescents is recommended for intramuscular administration. Intramuscular administration not only makes it easy to handle toxic states in cases of influenza, but also has a favorable effect on the clinical course of the disease, especially in cases of pneumonia.

In our opinion, the method of combined serotherapy should be considered as the best: the introduction of serum intranasally and intramuscularly. This recommendation is confirmed by experiments carried out in white mice infected with a strain of virus A2 which was adapted to these animals. The seroprophylaxis of Asian influenza proved to have optimal effect when the serum was administered intranasally within five days prior to infection. According to recent data, the prophylactic efficiency of the serum drops if administered earlier, and the serum has to be repeatedly used. At the present time anti-influenza horse serum is dispensed in the form

of powder (dry serum plus the Soviet sulfonamide preparation "Sulphademizin"), in the form of ointment, and a liquid (gamma globulin and convalescent serum).

Concluding Remarks

The problem of the successful control of influenza is one of the most burning problems of modern medicine. It is not only the direct effect of influenza itself on the health of man, but also the cardiovascular complications and other diseases concomitant with it, that call for the coordinated effort of scientists of many countries and especially of scientists of the Soviet Union and the United States.

To start joint work in this direction and to determine the best method of mass prophylaxis, we believe it essential to carry out broad observations and research on immunization against influenza with "inactivated" and "live" vaccines. The results of coordinated work of American and Soviet virologists in the field of poliomyelitis vaccination are highly encouraging in this respect. This noble cooperation must be continued!

Addendum

Some of the material referred to in this paper has been covered in greater detail in the following:

(1) Zhdanov, V. M.: Results of Further Research on Influenza in the USSR, Bull WHO, 1959, *20*, 261.

(2) Zhdanov, V. M.: The 1957 Influenza Pandemic in the USSR, Bull WHO, 1959, *20*, 489.

General Discussion

Dr. C. M. Mac Leod: I would like just once again to express our appreciation to Dr. Soloviev for coming to this conference. Now I have the happy task of turning over the discussion period to Dr. Thomas Francis. Unfortunately, I must go to some other duties.

Dr. Thomas Francis, Jr.: I suspect that part of the call away from this meeting is perhaps a muzzling effect, as the chairman is not supposed to talk except to introduce people. I understand that this is supposed to be free and open discussion at the present time. I would like to suggest that while we presumably now have forty minutes for general discussion of this afternoon's general subject, we should try to keep comments and discussions to a limit of about three minutes and let more people in on the fight—I mean the discussion.

Dr. Keith E. Jensen: I have heard it said several times that this conference is much too formal and that perhaps we have come together, if not to stimulate each other, to irritate one another, and several things have been said this afternoon that have irritated me. I have a list of them and I will try to stay within the three minutes.

First, the matter whether we have a rearrangement of antigens or a production of new ones: I do not think it makes too much difference really; it constitutes a threat when it happens just as it did very recently. Second, I am also struck by the very weak point in our theory that, as people get older and older, they develop a wider spectrum of antibody and thereby derive some greater protection in this manner. It seems to me we still have not done the definitive experiment, namely, to challenge these people in an experiment such as was done naturally in 1957. This would be to instill into these people with different antibody spectra a challenge virus that would be new to them. I would like to ask Dr. Salk whether he feels that the cycles he has demonstrated here are really the cause and effect mechanism that the rest of us have long believed them to be.

It is true that antibody builds up in a population and, when we see a change, how do we know this antibody actually effects that change. We know now that a very large proportion of our population has antibody to the Asian strain, and yet very few of us believe that a major shift is imminent in the next few years. It seems, rather, that we might have a ten-year cycle—at least that is what we had in the past—and this does not relate to the necessity of building up a virgin population such as we see in the measles picture.

The matter of toxicity of the vaccine has been played down a bit this afternoon. I think in reality it constitutes a problem. Pediatricians hesitate to use influenza vaccine because it does often cause a reaction in the order of 10 to 20 per cent in age groups from six to sixteen. One other point, and that is the rather curious effect we noted when we were using two different polyvalent vaccines—one a four-strain vaccine and the other a six-strain vaccine. Namely, an advantage accrues by using the six strains in that one gets an increased amount of antibody or increased incidence of antibody production to any one of these strains. This is reported in a paper that will soon appear in the *Journal of the American Medical Association*. With this I relinquish the microphone.

Dr. C. H. Andrewes: Dr. Francis, I very much enjoyed all the discussions this afternoon and I only want to talk about one point and that is the value of polyvalent vaccines. Dr. Davenport referred to the view with which I think you are familiar about the cycling of the limited number of antigens. This is an unproved hypothesis. We in Britain, at least in Sheffield and Mill Hill, have been wholly unconvinced by this hypothesis. Another, different, view which we hold is another un-

proved hypothesis. But the difference between these two unproved hypotheses is this: on the basis of the Ann Arbor hypothesis the doctrine has arisen that it is a good thing to make your vaccine polyvalent. The stark fact is that all successful vaccinations that I have ever read of have been done using an antigen made with a current strain. Therefore, I am not convinced by any evidence that I have seen that the addition of any other antigens to it has done any good.

Dr. Davenport does not like the idea of a succession of crash programs and the dark future which this holds out before him. Unfortunately, the world has not been made to suit Dr. Davenport's convenience or mine, and I suggest that it would be very much better to face the world as we see it and not behave like ostriches and hide our heads under a stockpile of obsolete antigens.

Dr. Joseph J. Quilligan, Jr. (Los Angeles, California): Dr. Jensen raised the question of the toxicity of the vaccines, particularly in children, and I have some experience in this direction. I think we can minimize the toxic effect by giving large numbers of small doses of vaccine. We have given a group of children in California four doses of vaccine. The first one was monovalent Asian, containing 400 CCA units, and the subsequent three doses were polyvalent and again 400 CCA units against the A type and 200 CCA units of PR8 and PR301 and 100 CCA units of Great Lakes B. After the first dose of vaccine in the children, the experience we had was pretty much like what Dr. Meiklejohn said, we got essentially no antibody response. Part of the reason for continuing to give the four doses of vaccine was simply to see whether with each successive dose we might not be able to boost the A2 antibody titer, but we were rather unsuccessful all the way through the last dose. However, in January we had an outbreak of what apparently was A2 influenza. In our control group in the twenty wards where the 113 vaccinated were spread out, we had 1,221 patients, 384 of whom had what appeared to be an influenza-like illness, giving us a 30 per cent attack rate in the nonvaccinated group. We had 15 cases of influenza-like illness in our 113 vaccinated, giving us a 13 per cent attack rate. This was associated with rather poor levels of antibody even after four doses of the A2 component, but excellent levels of antibody against the other A and B components of the vaccine. In addition to this, the levels of antibody rose each time we vaccinated the patients, and this is a little bit in contradistinction to Dr. Meiklejohn's observations. I think we have seen this before, too. One other thing about our observations, the vaccinated subjects were divided into those who received 0.1 ml. and those who received 0.5 ml. each time they were vaccinated. We consistently showed, following each dose of vaccine, at least (or approximately) a twofold greater antibody response in the 0.1 ml. group than in the 0.5 ml. group. When the patients were analyzed from the point of view of clinical protection in the 0.1 and the 0.5 groups, the numbers were so small it was impossible to come to any conclusion. There were more numbers of ill children in the group which received the 0.1 dose than the 0.5 dose, but they don't stand up statistically, whereas these other data I have given do stand up.

I would like to bring up one other thing—or perhaps Dr. Francis would like to discuss this later—and this is the study we did when we gave the monovalent PR8 vaccine to the children in 1946–1947 and when we were able to show that those children who received the monovalent vaccine prior to the outbreak of the A1 epidemic had antibodies following five doses of vaccine against the A1.

Dr. Joseph A. Bell: I would like to comment on the apparent paradox between the protection that Dr. Rose achieved and the lack of production of hemagglutination-inhibition antibody. The requirements for the National Institutes of Health are such that the vaccine manufacturers have to use a potency test that requires the immunization of mice against the specific strain, and it occurs to me that the mouse protection studies may be more in line with the protection possible in human beings than may be demonstrated with the hemagglutination-inhibition antibody test. All during the progress of these meetings I have noticed that the entire emphasis has been placed on the complement-fixation test and the hemagglutination-inhibition test without recognizing other types of information that are available to us, such as neutralization data and the protection studies.

Dr. Francis: I might comment that in some of the data published by Dr. Soloviev and his colleagues, they did do neutralization tests for serum specimens which gave very low hemagglutination inhibition and found that they had considerably higher levels of neutralizing antibodies in the mice.

Dr. Bell: Of the inactivated vaccines, the mineral oil adjuvant vaccine offers the greatest promise because of the high antibody response and the long duration of antibody and the demonstrated protection. We have had perhaps the most extensive experience with the use of oil adjuvant vaccine in general population groups which include young children, and I thought you might be interested in the occurrence of untoward reactions.

The occurrence of these reactions was primarily in Negroes and in children and for the most part took the form of post-vaccinational cysts. Monovalent vaccines of 100 CCA units each were used and we made a very determined effort to inject

them into the belly of the triceps muscle. We made observations over a five-year period from 1951 to 1955. There was a total of nearly 11,000 people who were vaccinated with these oil-adjuvant vaccines. The occurrence of post-vaccinational cysts which drained or required surgical intervention was 0.8 per cent in 1951, 0.1 per cent in 1952, and less than 0.1 per cent in 1953 to 1955. As Dr. Salk told you, a purified emulsifying agent was used in the years 1952 to 1955. It is interesting that only 2 per 1,000 occurred in the total experience, or less than one per 1,000 after 1952.

In 1951, we vaccinated both Negroes and whites, and in 1952 to 1955 we vaccinated only whites. The Negroes had the highest percentage of reactions; the reactions in whites were significantly less. You will note that for both groups the proportion of reactions was much higher in the very young children than in the adults. The reaction rate for adults is about the same as was observed in military groups which had been vaccinated with the early adjuvant vaccines. An important point, I think, is that particular attention was paid to giving the vaccines intramuscularly by the few vaccinators using them. If the vaccines were to be used in the general population, more vaccinations might be given subcutaneously and possibly result in even more of these reactions. However, the adjuvant vaccine is the most promising vaccine product we have so far for inducing antibody response and giving protection over a long period of time. I think we have to bear in mind that in young children we must be cautious in its general use, but certainly it is promising for adults.

Dr. Joseph W. Cooch (Washington, D. C): I would like to point out that the Army has continued to use vaccines since 1958. However, we are continually reviewing the recommendations each year and hope the most current advice is given us for the armed services. One statement has been made a couple of times which I question: that there has been no influenza in the military this year. We have had spotty outbreaks. We are not sure yet how much of it is influenza, although both at training posts and at permanent stations, we have isolated influenza viruses. The number of cases on which isolations have been attempted is considered small as yet. We will not know until the end of the season how much influenza we have had; but we are not completely without it, although it has been very spotty. In California there has been practically none, but in posts in South Carolina and in the Middle West we have had some rather sharp outbreaks of respiratory disease.

Dr. Florence S. Lief (Philadelphia, Pennsylvania): I would like to add another hypothesis, or variant, as long as this meeting has been devoted to variants, of the hypothesis Dr. Andrews referred to. It is a variant of Dr. Davenport's hypothesis: that influenza viruses contain all the antigens and that because of this, recycling is inevitable if the influenza viruses are to survive. This implies that there are a small number of antigens present and that the antigens that appeared dominant seventy to eightly years ago have been the cause of the current outbreaks. However, one also can conceive that there are not an infinite number, although a great number of antigens and that most of them have been present in trace amounts in the variants over the years. One of them, we believe, is the A2 antigen. The guinea pig experiments that were referred to this morning and the data on human sera, we believe, are indications of repeated exposure to very small amounts of antigens over a period of time, which finally produced A2 antibodies. This brings us to the question of vaccination. If repeated doses of inactivated vaccine material are given, containing tremendous amounts of hemagglutinins, we find we cannot broaden the antibody spectra. We get strain-specific sera. However, with live virus, in which all antigens might be present but in varying amounts, repeated exposure to growth of virus *in vivo* allows enough of even trace antigens to be available for the production of antibodies. That, of course, suggests that the answer to a vaccine which will protect against current and future strains might be one which the Russians are using at this moment, namely, a live one.

I would like to make one more comment. I shall try to explain the difference in findings of those of Dr. Stuart-Harris and ours. In our series of vaccinees who received A2 vaccine, there were 128 subjects, ranging in years from eleven to sixty and more. They were divided into four age groups. Those more than twenty years of age gave the type of discrepant responses he referred to. In the younger age group of eleven to twenty, however, only 27 per cent had antibodies to swine and 50 per cent had antibodies to PR8. We found that the chief increases in these antibodies to A2 vaccine were in individuals who had pre-existing antibodies. In other words, there was rare conversion from negative to positive. Dr. Stuart-Harris, if in that group of twenty-five individuals whom you tested —(now I hope they were less than twenty years of age. Were they? Yes. It was the school you described yesterday? Yes.)—you have a statistical possibility of having some who would not have a baseline of antibodies to show the increase following vaccination. Is that plausible?

Dr. Fred M. Davenport: This [microphone] is a live one—not a vaccine but a live instrument of torture. I didn't come here to irritate anybody. I can be irritated at home, or irritate people by staying home. I agree that the comment made by

Dr. Andrewes that we must face the world as it is, not as we would like it to be, is a real one. It certainly is a cogent one. I would like to take exception to the statement, however, that he made that there is no evidence that vaccines not containing a recent strain are ineffective despite the implication that there was concordance in the views of Mill Hill and Sheffield. The example that I was quoting in the paper, and actually Dr. Stuart-Harris showed the slide, was of protection in 1955 using as its latest A1 component a strain isolated in 1947. If my arithmetic is correct, this represents an eight-year interval and that is not an endless series of frequent crash programs. I can provide the reference if need be.

In our own experience, in the spring of 1957, the influenza A1 strain in a monovalent vaccine was actually isolated in 1954. That is a three-year interval. I am much more concerned, rather than swapping witticisms, with the fact that facing the world as it exists, we were talking about an excess of 80,000 deaths. Now dead people are dead people. They can be counted. They can be studied. They can be revered and lamented. But if we have at hand, as I believe we do, one or more alternate methods of preventing such deaths, our major job as physicians is to take effective action to prevent those deaths. We can continue to enjoy the debate for the next thirty-five years.

Dr. Jonas E. Salk: I suppose that the fundamental question about two unproved hypotheses is, how useful is each one. I would be tempted to ask Dr. Andrewes whether, on the basis of his hypothesis, vaccination should be practiced at all. As for his reference to stockpiling, I do not know that anyone would consider stockpiling, but rather using the vaccine that is available. I believe that the basic concept here is to construct a vaccine composed of all of the antigens known heretofore and to use that in an intelligent way, which would mean to provide such basic immunity to all individuals as they are born. New strains would be added to that basic vaccine and, if a new variant appears, admittedly, it would then be necessary to re-vaccinate all who have previously been vaccinated, and those still to be vaccinated for the first time would then receive the basic material to which the new component was added. Now this seems to me to be a rather orderly progression of events.

Admittedly, the cycling hypothesis—and this is an answer to Dr. Jensen—has not been proved, but, if in time this is proved, then one would have acted on the basis of an hypothesis that ultimately may be supported. I think that the evidence is clear, as Dr. Davenport has shown very conclusively, that for a number of years, vaccines certainly have done what they were intended to do.

Dr. Andrewes: May I just answer the last two speakers? I do not think, really and truly, that Dr. Davenport and I are in so much disagreement as it might appear. The instances he gave of course apply to the viruses within the A1 group, and I think we all of us agree that we can get a great deal of cross immunity and cross protection provided we keep within the A1 group. I'll give him that one. But I hope he will allow me to say that the jump from A to the A1, and the jump from the A1 to the A2 puts us in a position where our current vaccine is pretty useless. And as to Dr. Salk, I certainly do not advocate that influenza vaccination should not be practiced at all. I am all in favor of influenza vaccine as a scientific method of controlling influenza, but you have got to use the right one. Let us take an example: at the present day, we have this A2 virus. I don't see any point whatever in adding any A or A1 to a vaccine containing A2. I do not know of any evidence that that would do any good at all; I think it is just a waste of good A and A1 viruses to put them in.

Dr. C. H. Stuart-Harris: Dr. Francis, there is one aspect of vaccination which I think has not been stressed quite enough this afternoon. I believe we ought to draw a distinction between control of a disease and protection of individuals. It does seem to me that all we have heard, including even your own words last night about the extraordinary difficulty in producing enough active vaccine, even when one does have a couple or three months to spare, should make us hesitate before we think that by immunization we really are going to gain control over an infectious disease with a spreading capacity even as limited as Asian influenza. On the other hand, with regard to persons at special risks, that is a totally different matter in my viewpoint. I have yet to see, actually, any figures published anywhere in which it has been shown that immunization will prevent death. These 80,000 excess deaths that Dr. Davenport referred to, were, in fact (if the figures in the United States are at all parallel with those in England) drawn largely from persons more than fifty-five years of age in the community. These are the ones who are supposed to have (in fact, according to your own definition) the widest possible degree of immunity; and yet these are the ones that die. I think we have to be very careful how we think about this whole problem.

Dr. Quilligan: We had some experience with deaths in 1957 and 1958 at the Los Angeles County Hospital. There were somewhere in the neighborhood of a hundred of them, and approximately a third were children of three years of age or less. The pattern of their deaths was fairly rapid. I do not recall the figures exactly, but I remember that,

within this group of children, two-thirds had illness lasting only a total of three or four days.

Dr. Thomas G. Ward (Washington, D. C.): This is one aspect of influenza control that I do not think this conference has stressed enough, and I would like to bring it up, partly for the sake of completeness. It has been suggested that Asian influenza A2 virus vaccine is not effective. However, this is probably not due to the fact that it will not produce the antibodies, but because people do not take it. Actually, I wonder if this conference might address itself ultimately to the question of whether vaccination is really the correct way to control influenza at all. I think the evidence is quite clear that people will not be vaccinated in sufficient numbers really to reduce the disease sufficiently to justify all this effort. In spite of the urging of health authorities to be vaccinated, people simply do not take it very seriously. I wonder if really we should not attempt to set up a technique by which we can engineer, in some fashion, the virus into the human nose. Now there may be a battery of systems by which one might do this. Possibly the live virus could be given to large groups of people in motion picture theatres by spraying it through the air ducts, or some type of technique might be devised by which one could offer people a pill which would change the receptor sites on the surface of the cells in the lining of the nasal mucosa or in some fashion change the receptorability of the human nose. I do not think vaccination is going to work because we cannot get enough people to take it.

Dr. Francis: It seems to me, if I may talk for a minute, that one of the points that is being overlooked in much of this discussion is as if this all *had* to be done on an emergency basis, or else. This relates to the hypothesis of the recurrence of dominant antigens, and that there are multiple antigens in most strains whose expression can be demonstrated. I think our basic objective should not be simply to see how fast we can do it for a limited number of people, but I am wondering if influenza should not be considered appropriate for a basic immunization beginning in childhood, as Dr. Bell suggested. One could then build onto this immunity by introducing new antigens and building a broad spectrum of antibody. I think that different hypotheses should be used simply as guidelines for work, and not considered in any way mutually exclusive. In my own mind the idea of getting as broad an antibody base laid down as early as possible and then building onto it so that it gets still broader and broader with increasing age or as age goes on seems to be a very reasonable working hypothesis. I would disagree with the idea that one should do something only on an emergency basis without doing something that may have a broader foundation and a more continued long-term effect and which can be reinforced as needed. This is what we do in practically every other form of vaccination that is being used in childhood at the present time.

Now, to go back to the question of whether vaccine is going to be the eventual answer, or whether we are going to find other things; these again are problems for study. These are the things that this conference is supposed to be helping to emphasize: that the treatment of disease or other ways of arriving at preventive measures are really important areas of study. We would be very dull to conclude a discussion of this sort on the basis that it is either/or, or that we should draw some sort of concurrence which in any way would stifle investigation along any lines.

I think it is our view that vaccination is the thing that we can use at the present time. It is a thing that we can demonstrate as effective. Whether everybody who gets vaccine fails to get influenza under all circumstances I don't think matters at this stage. Obviously, we are very "wet behind the ears in this field," and we have to learn as things go on; but I hesitate to think that a conference of this sort would end up by suppressing ideas that could be followed so long as somebody wants to prosecute them by forcing some other line of discussion or some other line of approach out of consideration. And I would like to quote our friend T. S. Eliot, who said, "The only kind of wisdom we hope to acquire is the wisdom of humility." And perhaps that is what we need now.

Dr. James P. Leake (Washington, D. C.): I don't know if Dr. Karsner is still here or not, and I don't see anyone else that I am sure was very active in 1918. I cannot help remembering those bloody lungs. I unfortunately did not hear Dr. Dauer's paper, but to my mind there is no question but that the total mortality is the best index of influenza outbreaks. In that total mortality (Dr. Davenport speaks of saving lives) a great proportion of deaths occur in the older age group. I just wanted to propose more study of the swine influenza virus group (that Dr. Francis believes was active in 1918) as to their effect on blood vessels and blood coagulation.

Dr. Francis: Thank you, Dr. Leake. I am sorry you were not here yesterday because this was part of the program that was under discussion, and I think with a good deal of interest.

There were two other questions that were asked that nobody seems to have picked up. One was Dr. Jordan's as to whether it is necessary to inactivate influenza virus for subcutaneous inoculation, and the answer is "no"—at least from the point of view of infection. This we demonstrated at least with tissue culture virus in the first studies we carried out, both by subcutaneous and intracutaneous in-

oculation of active virus. We actually gave it to some people who had colds at the time they were given it to see whether there was any effect, and it provoked no disturbance that could be observed. From the factual side, however, it might be a much more difficult problem to handle in actual practice. I am sure Dr. Murray would vouch for some of the difficulties that might occur.

The second question that was asked was about the occurrence of influenza in the military. I think that Dr. Rose and Dr. Meikeljohn and Dr. Lennette did emphasize the fact that, whether it is occurring or not, there appeared to be a very distinctly favorable effect of the vaccine in prevention.

Dr. Jensen: Just one more comment. And this is to dwell again on the differences that are necessary in our thinking as to whether we are going to protect every individual—for example, someone in an added health-risk group, or someone in the military, or, on the other hand, to attempt to protect the community and school children. It seems to me that we require here two sets of requirements for vaccine. What is the smallest amount of vaccine that might do some good in a community? Early in 1957, we set some rather arbitrary limits to potency of vaccine in terms of chicken cell agglutination units. I would suggest then that now is the time to attempt to get some definitive information as to what is the smallest amount of vaccine necessary to confer immunity. Perhaps this information could be obtained by giving vaccine in multiple doses, parenterally, to a group that has not had previous experience with that particular strain. An example would be swine influenza virus given to children.

Dr. Francis: I think that what Dr. Jensen has said has emphasized the fact that many of the things that are being discussed are still experimental and they should continue to be experimental. There is a great need for more knowledge. I think it would be fine if Dr. Jensen would like to develop that kind of study, because it won't be an easy one. Nevertheless, to say that these things are needed means that somebody is needed to do them.

Thank you. The afternoon session is adjourned.

ROLE OF HEALTH ORGANIZATIONS IN THE FUTURE

JOHN H. DINGLE, *Chairman*

INFLUENZA PANDEMIC: PREPAREDNESS PLANS OF THE PUBLIC HEALTH SERVICE

L. E. BURNEY[1]

Introduction

First, may I express my gratitude to the Advisory Committee on Influenza Research and to Dr. Loosli and the members of the Asian Influenza Committee for their fine work in conceiving and arranging this excellent program. The function of the session today is to take cognizance of the knowledge acquired during the 1957 Asian influenza epidemic, and all preceding experiences, in order that we may develop in this country a reasonable plan of action for a future pandemic of influenza. It goes without saying that knowledge and plans which may be helpful for a pandemic also will be of value in handling such a situation as now faces us when we are having numerous local outbreaks of influenza throughout the country.

Since the great pandemics occur at intervals of a generation or so, and since it is human nature to forget the disagreeable things of life, it is unfortunately true that we have approached each pandemic almost as if it were an entirely new problem. Our objective at this conference is to crystallize the recent experience and to file the information in an available place (without forgetting where) in order that the next generation can at least begin its battle with the best plan that its predecessors could create.

The experience in the 1957 pandemic of influenza, which happened to originate in Asia, was unique in the history of this disease. For the first time, an epidemic was promptly recognized as being caused by a strain of influenza which differed significantly from those with which man had had any experience for the past seventy-five years. The build-up of this epidemic into a pandemic was followed, step by step, and its progress throughout the world was carefully plotted. Unlike previous pandemics, this one was identified by virologic procedures as well as by clinical and epidemiologic observation.

Like the other speakers this morning, who will tell you what their organizations propose to do when the next pandemic reaches us, I shall spend most of my time outlining the preparedness plans of the Public Health Service. The efforts of the Public Health Service will, of course, be integrated with those of other groups in the United States and in the world. Our efforts will likely fall into four broad categories: (*1*) recognition of the pandemic; (*2*) preparation of vaccine; (*3*) use of vaccine, including problems of vaccine distribution and dissemination of information; and (*4*) research.

Recognition of Pandemic

I shall not belabor the need for improving the reporting systems throughout the world which serve as tracking stations for following epidemics of whatever cause. The Public Health Service's reporting system comprises our foreign quarantine division, with its officers stationed in various parts of the world, and its liaison with private organizations such as transportation companies, plus our state and national health reporting systems. This network is integrally related with that of the World Health Organization. We shall strive to improve these services for the continuous monitoring of various types of infectious diseases. These improvements will automatically strengthen the total reporting system when pandemic influenza next occurs.

Since the beginning of World War II, the influenza diagnostic laboratory facilities in the continental United States and in U. S. military installations overseas have constantly improved. The essential diagnostic procedures are no longer limited to virus research laboratories. On the contrary, they are broadly available in the laboratories of our cities, our states, and our overseas bases. All of these laboratories are ultimately tied in with WHO surveillance system, and about seventy-five of them receive immediate support from the International Influenza Center for the Americas, which is established within the Public Health Service and is located

[1] Surgeon General, Public Health Service, United States Department of Health, Education, and Welfare.

at the Communicable Disease Center in Atlanta, Georgia.

The Influenza Center maintains periodic correspondence with each collaborating laboratory, determining its current facilities and its need for standard influenza diagnostic materials which the Center prepares and supplies. The Public Health Service is proud of the constellation which includes the Influenza Center and the collaborating laboratories in state and city departments of health and in university research centers.

We shall strengthen the facilities of the Influenza Center in order that it may increase its program of technical training in influenza typing and diagnostic procedures and augment its capacity to supply the collaborating laboratories with diagnostic materials. In addition, the Center and its associated laboratories must remain in readiness to initiate promptly, and carry through to completion, antigenic studies on newly isolated strains of influenza and serologic assessment of immunity of a population. The latter was of great assistance in 1957 and will be invaluable in the future in predicting whether a local epidemic, wherever it occurs, is likely to progress into a severe epidemic among our people.

Over and above this, we must be prepared to put into the field small, mobile teams which will be able to establish the precise etiology of influenza outbreaks wherever and whenever they occur and to return to their base laboratories with strains and materials that can be used for production of diagnostic reagents and vaccines.

Finally, we shall maintain a competent advisory group which will stay abreast of all types of information on influenza and which can advise the Surgeon General when a local epidemic shows possibilities of becoming a pandemic.

Preparation of Vaccine

In discussing the recognition and surveillance of the pandemic, I mentioned that the experience in 1957 was unique. I would be justified in using the same term "unique" to describe the development and production of more than 50 million doses of Asian influenza vaccine within seven months after the virus was first isolated by workers at the Walter Reed Army Institute of Research. In reviewing this record, it is difficult to see where time might have been saved. Each step, from the recognition of the outbreak in Hong Kong to the preparation and distribution of the final vaccine and the supplying of information to the public and the medical profession, was taken at top speed, with all available resources mobilized. I think that everyone who participated in this effort can take pride in this outstanding achievement.

Had the main force of the epidemic struck late in the fall, as we expected on the basis of previous experience, this major effort in which the pharmaceutical industry played so important a part would have been even more successful. Despite the early arrival of the epidemic, there was some vaccine on the market before the first local outbreaks were reported. But only 4.6 million ml. were available by the fifth of September, when the epidemic wave was breaking. By the first of November, when 42.6 million ml. were available, the week of highest incidence had passed. Thus, the supply was limited during the period when it could have been most useful.

No one can reasonably deny, however, that the production schedule was impressive, nor that the vaccine which was used had an effect in reducing the impact of the epidemic. It is easy to enumerate quickly certain of the essential points which made the achievement possible. First was the prompt isolation, in a foreign land, of an agent producing an epidemic which did not reach our shores immediately and which developed into epidemic proportion here only after several months. Second, the fact was quickly recognized that our people did not possess antibodies against this hitherto unknown strain. A third factor was the continual interchange of scientific and professional information between the governmental agencies and the vaccine producers; an indispensable fourth was the full cooperation of the manufacturers, including their sizable investment of risk capital. Finally, the decision was made, long before the disease reached epidemic proportions in the United States, to use a monovalent Asian strain vaccine standardized for potency by a simplified, rapid technique which was employed simultaneously on each batch by the Division of Biologics Standards and the manufacturer.

It would be wishful thinking to assume that, when the next pandemic invades the United States, we will be so fortunate as we were in 1957 in having a long lead-time. Further, it seems unlikely that the same kind of approach used in

1957 would appreciably decrease the interval of four months which elapsed from the time the new virus reached American laboratories until 5 million ml. of vaccine were delivered for use. Therefore, we must find alternate approaches if we are to succeed in producing a vaccine in the teeth of an epidemic.

One of the significant time consumers in 1957 was the adaptation of the strain to the point where it would provide crude material sufficiently rich in virus to produce a vaccine containing 200 or 400 chicken erythrocyte agglutination units of antigen per ml. Should the next pandemic be caused by a previously known strain, or by one with an antigenic complex that can be stimulated by using a polyvalent vaccine containing several laboratory strains, each of which has one or more antigens of the new pandemic strains, then the time can be shortened appreciably. If only the efficiency of a given unit of antigen in eliciting antibodies and immunity in man could be improved, then smaller quantities and less potent material would be required in order to get vaccine production under way. It is to be hoped that our research efforts before the next pandemic will provide a solution to this problem. At the moment I would not hazard a guess as to whether this solution will come through the use of adjuvants, through the use of live virus rather than the killed material used in 1957, or through new methods of immunization.

Use of the Vaccine

Whether or not a vaccine is available in time to immunize the population in advance of a spreading pandemic will depend upon chance as well as on thorough preparation. In any case, we can assume that available stocks of vaccine will be in extremely short supply during at least the initial phase of any preventive medicine operation. Consequently, advance planning must provide lists of types of persons who should receive vaccine on a priority basis. These categories of persons will fall into two general classes. One will include those who might be considered medical risks and whose immunization might prevent death, among whom will be the aged, the debilitated, and pregnant women. The second will comprise those persons who should be kept healthy because of their essentiality to the nation and the community. Included among the last group are persons in the military services and, in the civilian community, those concerned with preventive medicine, the care of the sick, communications in all forms, transportation, utilities, fire and police forces, and certain essential industries.

I am happy to report that the planning of the Public Health Service, as part of an exercise during the influenza epidemic of 1951, provided a list of essential civilian occupations which included some six million Americans, and this list was promptly put into use in the planning for the 1957 Asian influenza outbreak. In the future, we should keep such lists up to date and, in addition, prepare comparable estimates of the numbers of people who belong in the medical risk categories. As an ancillary value, such lists would provide the manufacturers with an estimate of the minimal amount of vaccine which would be required in an emergency.

Such a conference as this must not only recognize successful procedures and arrange for their continuation and augmentation, but also acknowledge deficiencies in order that they may be corrected. I regret to say that our experience with the distribution and use of influenza vaccine during the early days of Asian influenza in this country left much to be desired. After careful consideration and with the advice of health officers and of industry, the decision was made to distribute the available stocks without restrictions. Dependence was placed on informing the health professions and the public of the categories of persons who should receive vaccine in the first priority. The manufacturers cooperated in 1957 by distributing their stocks to the states on the basis of population. But beyond this point the priority system in many instances was not followed.

We have time before the next pandemic to work out with the various health groups concerned a better method for voluntary control of influenza vaccine when it is in short supply. I feel confident that plans which promise to meet the problem will materialize and that the necessity for compulsory allocation will consequently not arise.

One of the significant contributions to knowledge gained during the 1957 epidemic was the demonstration that success or failure of a vaccination program in a given community was largely dependent upon the attitude of the local medical society and its individual members. It is not enough to have fine policies and words

from the federal and state health officials and the national medical and health organizations. Unless the local medical community is informed, and ready and willing to act, any program seems doomed to failure.

In developing plans for informing and educating practicing physicians, cognizance should be taken of the difference in points of view of the physician and the health officer. The physician is primarily concerned with his individual patients, particularly those in the medical risk category, and what the vaccine will do for them. In contrast, the health officer looks at the community and aims his immunization program at the health protection of those segments of the population whose continued activities are essential for the community. We cannot expect to change the points of view of the physician and the health officer, but we can hope, by appropriate educational measures, to help each understand the other.

An important corollary to the information that reaches the public through its medical and health authorities is the information that reaches them through lay sources: the press, radio, television, magazines, meetings of clubs, and business, church, and other groups. If our story is clear, convincing, and told in simple, specific, and understandable terms, it can and will be quickly disseminated through national channels. A public opinion survey, conducted shortly after the 1957 Asian influenza story broke, indicated that almost 90 per cent of the people had heard about it.

But hearing about it obviously is not enough when specific action is desired. Like the professional public, the general public must also get information from local sources before it is ready to act. Here again, failure to develop effective local information programs has been our Achilles heel. It is unrealistic to expect that all local medical societies, health departments, and other key agencies will be staffed with information specialists who can team up to organize local promotion programs. However, much can be accomplished when representatives from the local press, radio, and television stations are included as members of the local planning committee. Too often, I fear, they are not only omitted from committee membership but even barred from covering the planning meetings.

We must keep constantly in mind that it is only the local channels that can provide information about the availability of local supplies, the time and place of clinics, the priority that will be given to various classes of eligible persons, and the other details that people must have before they can act effectively. The Public Health Service will therefore give primary attention to ways in which state and national agencies, working cooperatively, can help communities develop strong information programs.

Research

What I have said thus far has largely concerned the practical aspects of the influenza problem—how best to apply our present knowledge and resources in combating epidemic disease. As this audience well knows, significant progress against influenza in the years ahead will depend on how successful we are in shrinking our present areas of ignorance.

The importance and urgency of influenza studies are, I trust, evident in the priority given this area by the Public Health Service. We have created a National Advisory Committee on Influenza Research. And we have set up a Subcommittee of Investigators to enable the parent group to operate with maximal efficiency and dispatch. We look to the Subcommittee to stimulate and maintain long-term research interest in influenza, to assess research needs, and to help establish the general climate which is indispensable to productive study.

The major gaps in our present knowledge of influenza are painfully evident to all of us. We shall foster research on the mutagenic capacity of influenza viruses, on the nature of the virulence factor, on detailed analyses of the full spectrum of antigens of all known prototype strains of the viruses, and on the pathogenesis and physiologic abnormalities of the disease in man. In brief, we shall look to the Subcommittee of Investigators to encourage basic studies on the virus and on the abnormalities it produces in man in order that this knowledge may be used during the next pandemic.

In the area of applied or developmental research, we should sponsor research on chemotherapy and chemoprophylaxis of influenza and improve the methods for lengthening the protective period afforded by vaccines. In the latter category, research should be pushed on the development of adjuvants—materials which, when added to vaccines, are capable of greatly boosting the antibody levels produced by immuniza-

tion. Experimental results to date are sufficiently encouraging to warrant extensive exploration of the whole problem.

Many other areas might be cited in which research could profitably be initiated or expanded. I will mention only a few: the use of germ-free animals to explore the relationship between single bacterial infection and influenza virus infections; neurologic research on the causes of myalgia and post-influenzal myasthenia; and studies relating to the cardiopulmonary complications common to influenza. These examples and many others one might name suggest that influenza is still very much an unfinished business.

The kinds of research I have in mind, and which we shall foster, will be valuable not only for the scientific knowledge acquired but also because they will provide a nucleus of intelligent, trained people who will be available at a moment's notice to provide the Surgeon General of the Public Health Service in 1980 with the kind of essential advice that he will require if faced with a pandemic of influenza.

In preparing the legacy for my successor who will hold office when the next pandemic strikes, I hope to provide him with three different kinds of sound investments. There are: (*1*) a finite set of operating plans based on the experience of 1957; (*2*) a continually accumulating body of scientific information resulting from sound research on influenza carefully fostered by a dedicated cadre of investigators; and (*3*) a group of senior scientists and health officers who have maintained an acquaintance with the field and who can serve as his advisors.

Conclusion

These actions, if carried out in the inter-pandemic period, will be of great value not only to the United States, but to other countries as well. Information which we obtain from our state and national surveillance and from field teams outside the borders of the United States as well as from our research laboratories and biological houses within the United States, will be of as much value to others as to ourselves. Where the next pandemic will start no one knows. If it starts in our own country, we would nevertheless expect to accumulate—and disseminate—the information necessary for effective control measures, even though it would be obtained too late to be of value to us. We would do this because it would help others—a motive that is a tradition of our nation and the Public Health Service.

THE ROLE OF MILITARY MEDICAL SERVICES IN FUTURE EPIDEMIC INFLUENZA

JOSEPH H. McNINCH[1] AND R. P. MASON[2]

Introduction

The role of the military medical services in future outbreaks of influenza is visualized as one of basic similarity to that which the military services have played in the past. The program, we believe, will follow the principles of recognition and surveillance, control by use of vaccines and whatever other methods may be developed, continued research in laboratories and hospitals within the military services, and support of civilian investigators by collaboration and through research contracts. We will continue to train young men in diagnostic and research procedures so that a greater pool of such persons is available, whether they remain in uniform or not.

Surveillance

Since World War I the military services have had a great interest in influenza, but it was 1940 before an organized effort was put forth. On December 27, 1940, Lt. Col. James Stevens Simmons prepared a letter for the signature of the Surgeon General of the Army. This letter was addressed to the Adjutant General and requested authority for the Surgeon General "to appoint and maintain a board for the investigation of the etiology, epidemiology, prevention, and treatment of influenza and other acute epidemic diseases in the army." This board was to consist of "such prominent physicians and other scientists as may be required from time to time, regardless of whether or not they are in the military establishment or other federal services or civilians not in the employ of the Federal Government." This request was approved by the Adjutant General and was the genesis of the present Armed Forces Epidemiological Board with its various commissions, including the Commission on Influenza. Since that time the military services have been on the alert for a pandemic of influenza and consequently have had opportunities to study many epidemics of varying size and severity. During this time the services have cooperated and collaborated with the Influenza Center of the Public Health Service. We believe that, because of these years of preparation, the military services were able to play an important role in the 1957 pandemic and are equipped for equally important roles in future epidemics and pandemics.

In 1957 the 406th Medical General Laboratory, located in Japan, was able to send a team to Hong Kong to study the epidemiology of the disease among the civilian population there and to obtain throat washings for virus isolation attempts. At the same time, a United States naval vessel was returning from Hong Kong to a base in Japan and a few cases of influenza occurred on board. The 406th Medical General Laboratory isolated a virus which they recognized as being different from the known type A strains. The earliest recognized cases among United States military personnel, therefore, were in naval personnel, the isolation being made in an army laboratory, an example of real interservice cooperation. The new virus was immediately sent to the Walter Reed Army Institute of Research, where Dr. Hilleman began his well-known investigations.

The first significant outbreak among military personnel occurred at Clark Air Force Base in the Philippine Islands during May. Outbreaks of the disease followed rapidly in all of the services in Korea, Okinawa, and Japan.

The United States Army Medical Research Laboratory in Malaya collaborated with the British Army in studies in that area in both military and civilian populations.

Preventive medicine officers in the military services were able to follow and to predict outbreaks in these overseas areas and in military personnel and their dependents returning to the United States by sea and air. Information on epidemics in troops overseas and in the United States was transmitted to the chiefs of preventive medicine of the three services in Washington. These three officers maintained a constant communication with each other and, at the same time, a close contact with representatives of the United States Public Health Service and with civilian investigators, particularly those of the Commission on Influenza of the Armed Forces Epidemiological Board. This system of close col-

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laboration provided opportunity for information to flow in all directions.

The newly isolated strain of virus was provided to other medical research laboratories, both military and civilian, so that research on this new strain could move ahead as rapidly as possible. The virus was also made available to the National Institutes of Health, and they in turn made it available to industry.

This somewhat detailed—though by no means complete—review of the role of the military medical services in the 1957 pandemic has been presented because it illustrates the capability of the military medical services to assist in surveillance of future epidemics and pandemics. This capability has been present for many years, and in planning for the recognition and control of future epidemics it may be well to remember that United States military medical personnel are dispersed rather well throughout the world, with effective diagnostic laboratory services reasonably available in most areas.

Each year the chief of preventive medicine of the Army requests major commands to institute surveillance procedures for influenza. These measures consist of combined epidemiologic, clinical, and laboratory diagnosis, and the prompt reporting of outbreaks. In addition, regulations require the reporting of unusual disease outbreaks at all times. The Navy and Air Force follow a similar surveillance pattern.

Within the United States the armed forces have several well-equipped and well-staffed medical laboratories which are capable of diagnosing influenza by both serologic and isolation methods. A few of these laboratories are able to provide field teams for extensive investigation of outbreaks.

In addition, medical research and diagnostic laboratories are maintained by the Army and/or Navy outside the continental United States in Puerto Rico, Japan, Formosa, Malaya, Cairo, and Germany. The Army also shares a laboratory in Panama with the United States Public Health Service, but the virus capability here is entirely that of the Public Health Service. While none of these research laboratories of the military services have the study of influenza as their sole or even their primary mission, most of them are capable of performing diagnostic tests for influenza and of providing some surveillance of outbreaks. These laboratories also transmit information of unusual disease outbreaks to Washington.

In 1957 a combined team of clinicians and laboratory investigators was formed at the Walter Reed Army Institute of Research to move to any area that could be reached readily where a major outbreak among Army personnel was available for study. A detailed protocol was established to include physiologic, biochemical, virologic, and bacteriologic studies in addition to detailed clinical observations. Fortunately, the opportunity for such a study did not arise, but in future outbreaks such teams can be formed again. These teams will be able to conduct some of their research at the site of the epidemic, but they will send materials to their home laboratory as well. Many of the investigators will continue research on these materials after they return to the base laboratory.

Vaccine

Routine immunizations for influenza are accomplished within the military by utilization of vaccines obtained from commercial producers. Specifications for these vaccines are established by representatives of the military medical services after consultation with members of the Commission on Influenza of the Armed Forces Epidemiological Board. These specifications must also meet the requirements established by the Division of Biologics Standards of the National Institutes of Health.

The use of influenza vaccine within the three military services of the United States may not always be uniform in the future, but a general statement can be made that vaccine will be used. The responsibility of the medical services is to maintain the health of the military, and vaccines which show promise of preventing or reducing disease incidence will be employed. None of the medical services of the military has a preconceived or irrevocable opinion as to the type of vaccine which can and should be used. The desires of the military are simply to have a vaccine which is safe and which produces a broad and long-lasting immunity. Whether this vaccine with broad and long-lasting immunity will be an adjuvant vaccine, a live attenuated vaccine, or an improved killed vaccine, we would not hazard to guess.

Personnel of the military services are usually placed high on the list of those individuals who

are essential and must therefore be maintained in a healthy state. No problem is anticipated, then, in accomplishing the immunization of military populations. The military medical services are also responsible for a large number of dependents, and the immunization of these dependents will be in general accord with policies established by the Public Health Service. In this regard I do not believe we will experience the apathy described by Dr. Rosenstock. In the event that it becomes necessary for the United States Public Health Service to control the distribution of vaccine in a future pandemic, a program to provide vaccine to the military services must be established by agreement between the respective Surgeons General.

Research

The threat of pandemic or repeated epidemic influenza makes continued research a necessity for the military as well as for the civilian population. Within their capabilities the military medical laboratories will continue research toward the development of a more efficient vaccine and more rapid diagnostic procedures. Research in civilian institutions will be supported through the Armed Forces Epidemiological Board as it has been in the past. Outbreaks among military personnel will be studied wherever possible from the epidemiologic, clinical, laboratory, and therapeutic standpoints.

Summary

The role of the military medical services in future epidemic or pandemic influenza is the same as the role of these services in the past. We believe that we have the capability of providing surveillance in many areas of the world and we will provide such surveillance not only for our own protection but for the protection of others. Many of our laboratories have the capability of recognizing new strains, and we believe this capability should be and will be maintained. We will continue our efforts to prevent and to control the disease by the use of vaccines and whatever other methods we have at hand. We will continue to conduct research to improve these methods. We will continue to provide information concerning outbreaks of disease and concerning our research efforts to the United States Public Health Service and to civilian investigators.

INFLUENZA: THE ROLE OF THE STATE HEALTH OFFICER IN THE FUTURE

MALCOLM H. MERRILL[1]

It was forty-eight and one-half hours ago that I entered this room with high expectations of being able to resolve some of my frustrations in my approach as a state health officer to the problem of influenza. I can truthfully say now that I am much more sensitive to the problems before us, and I have greater hope of finding the solution to these problems.

I am encouraged to believe that significant progress has already been made in the control of influenza and certainly in the control of mortality from influenza, and that we might very well be on the threshold of progressive acceleration of this program. It was barely seven weeks ago that a well-informed science writer from one of our San Francisco newspapers called me from dinner one evening to report, "There is a story out of Los Angeles of a mounting incidence of acute respiratory illness, resembling influenza. What is the situation and what action are you proposing?"

What must a state health officer, or a local health officer, take into consideration when answering such a question?

First, is there really an abnormal incidence of acute upper respiratory illness? In California, for the past number of years, we have had what we think is a fairly effective surveillance network in which certain schools, industries, physicians, and hospitals throughout the state report to us through local health departments what is happening on a weekly basis. From this information, we are able to get some indication of what is happening among our fifteen and a quarter million people. We carefully analyze these data as they come in, in terms of our experience of past years, in an attempt to determine whether or not anything unusual is happening. And, in the incident of the telephone call referred to above, I was fortunate in knowing that in the Los Angeles and Fresno areas there was an abnormal incidence of acute respiratory illness, running about double the normal. So, I could say, yes, the occurrence of illness was something like double the normal incidence, but that we did not have any current indication of anything abnormal in other areas of the state.

The next logical question that the state or local health officer is confronted with is, "What is the etiology of the illness?"

It has been indicated here that possibly 50 per cent of these cases are due to one or another of some fifteen identifiable virus agents. This brought to mind the experience we have had through the past two decades in our studies and work with the encephalitides in our state. Some twenty years ago, we knew that in California five hundred to a thousand cases of encephalitis were reported by physicians in normal years, and somewhat more than this in epidemic years. We did not know the specific etiology of even a single case. Since that time, gradually, through the work of our virus laboratories, the participation of the Communicable Disease Center laboratory, and other agencies that have worked with us, we have been able to ferret out Western equine, St. Louis, polio, more recently Coxsackie, and ECHO viruses, herpes, and mumps; and we have been able to identify more specifically the post-measles encephalitis. As a result of this gradual accretion of specific information concerning these illnesses, we are now able to identify about 50 per cent of all the cases of encephalitis that occur annually in our state.

Are we not just entering the same kind of era as it applies to the acute respiratory illnesses, wherein it is becoming possible to identify the the specific etiology of these illnesses? This will help us in the diagnosis and care of future cases and, perhaps, in the prevention of future illnesses.

Now back to the question from the news reporter. Yes, we had influenza. We had identified it in our laboratories as Asian strain influenza or A2; almost simultaneously one of our local laboratories, the Los Angeles City Laboratory, which is also developing this type of service, had identified the virus. So, from two sources we had the definite information that this specific etiologic agent was present in our population.

The next question was, "How much of the current illness is due to this agent?" Here, we are pretty much stopped at the present time. All we

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can do is estimate: perhaps 50 per cent was influenza. In the future we hope that we can do somewhat better in estimating what is actually occurring in the population.

Another general question we must take into consideration is the past history of the occurrence of this agent in our population. We must be in a position to evaluate to some extent what is the immunity status of the population in order that we can predict what might happen. In this particular incident, we knew that influenza was occurring in the Los Angeles and Fresno areas, but there was no evidence of occurrence in the San Francisco Bay Area. This gave an indication that perhaps it had not really reached the northern and central parts of the state up to that time. So we had to evaluate the past as well as the present occurrence of the disease in the state and what the potentials might be.

A fifth general question: What is the severity of the disease? This is always a question with influenza. Is this something different, or it it a recurrence of the 1918–1919 episode? Or is this something that we have been accustomed to throughout the years? Even at this time, however, we had a clue to something unusual. There were thirty-five deaths reported from influenzal pneumonia the week ending January 9. The normal was fifteen to twenty at that time of year on a statewide basis. This was nearly double the expected deaths, and most of these deaths were reported from the Los Angeles area. In the succeeding weeks deaths continued to rise.

One must evaluate, in such a situation, the duration of the illness and be prepared to give advice to the public. What should they do now? Should they all rush to be immunized? Should they all go to bed? Should they all see their physicians? And, very importantly from the standpoint of the state health officer, what advice should he give his physician colleagues and his local health officers? And here again is an area where we need much more specific and accurate information at the time these episodes break.

And then comes the "sixty-four dollar" question. What do we recommend concerning immunization? Again, we must evaluate the stage of the epidemic. The past exposure of the public to this agent must be considered; we must take cognizance of the effectiveness of the vaccine, what can be expected from it; we must take into consideration the safety of the vaccine and the incidence of reactions to the vaccine; and here we must be cognizant, as was brought out yesterday, of the age incidence of the reactions to the vaccine; and we must also evaluate the age mortality rate of the disease. Keeping all these factors in mind, we must be prepared to give some information about who is to get the vaccine, and what dose to recommend; whether the route of inoculation should be intramuscular, subcutaneous, or intradermal. We must be cognizant of the vaccine supply and make sure we do not say something that is going to upset the continuous balance between supply and demand. We have been confronted with this problem in several episodes in recent years with reference to polio vaccine, and are very sensitive to this possibility.

In this particular situation, we thought we were being very cautious and very careful in following the line that we had indicated last October; that those people at special risk should be considered for vaccination, particularly those people outside the Los Angeles area where the epidemic had apparently not broken, pregnant women, and people with chronic illness. But in the course of the translation of the comments by the radio and television and some of the newspapers, some of these qualifications were deleted, and the public got the impression that everyone should be vaccinated for influenza. As a result, within ninety-six hours all pharmacy shelves and all physicians' supplies of vaccine were depleted, and we found ourselves in precisely the same spot that we had been in in connection with polio vaccine in several instances: no vaccine and a terrific demand.

These are some of the problems that a state health officer must face in the dilemma of what to do about influenza. I think this is enough to indicate that we have our problems in the practical application of the scientific knowledge that we expect you scientists to give to us on how to approach this very difficult problem.

In conclusion, just a few comments concerning the future role of the state and local health organization in relation to the acute respiratory illnesses. I have already indicated some of our functions in this role.

Certainly our function role is to assist in defining the problem. What is the cause of this morbidity and this mortality that are occurring from these acute respiratory illnesses? We must extend our activities in the field of laboratory identification, so that as time goes on we can more and

more clearly define the etiologic agents and describe their specific parts in epidemic situations. We must apply prevention through vaccination. As of now, all I can say is that we must limit ourselves to selective vaccination.

I think the time has come for us to give serious consideration to going all the way and promoting a basic immunization of children with the object of building the immunity to the known influenza viruses early in life.

Dr. Salk mentioned in the course of his discussions that the knowledge we have far exceeds the knowledge that is being applied. Yesterday, Dr. Davenport suggested that we should get on with the vaccination and do it expeditiously with real vigor. I am inclined at this moment to agree. I would hope, however, that we could have more unanimity on the part of the scientific fraternity before we make such a move because, to a public health administrator, such agreement is quite essential. We simply cannot experiment on our patient, which is the community.

THE ROLE OF THE PHYSICIAN IN AN EPIDEMIC OF INFLUENZA

JULIAN P. PRICE[1]

What should be the role of the physician—as an individual and as a member of his state and national medical association—in the event of an epidemic of influenza?

In answering this question, I will recount briefly what happened two years ago. Then, drawing upon the experience of that period, I will present suggestions which might be of help in the future.

When the threat of an Asian influenza epidemic confronted the country in early 1957, representatives of the American Medical Association met with the Surgeon General of the Public Health Service to study the situation. It was agreed that there was need for action. A special Committee on Influenza of the American Medical Association was appointed and this group, in cooperation with representatives of the Public Health Service and of the American Hospital Association, organized and promoted a national program.

There were two parts to this program: organizational and informational.

On the organizational phase, state and county medical societies were informed of the potential epidemic and the effect which it might have on existing services and facilities, and they were urged to develop stand-by plans to cope with the problem. It was suggested that there be an evaluation of professional resources with plans for mobilization of all physicians, regardless of the type of practice, and utilization of all available nurses, nurses aides, pharmacists, laboratory technicians, and other health personnel. The Committee requested that provision be made for full use of hospital beds, with curtailment of admission of patients for elective surgery and diagnostic studies if necessary. Since there would undoubtedly be a shortage of vaccine, it was suggested that its allocation be made on the local level by local groups or councils. Above all, the Committee urged that state and local programs, sponsored by medical societies, be closely related to the activities of state and local health agencies.

The informational part of the program was extensive. Packets of material relative to the work of the Committee were sent to all state and county medical societies, to the chairman of state emergency medical service committees, and to the national offices of allied medical and health associations. A series of articles relative to the biology, epidemiology, clinical characteristics, and therapy of influenza was published in the *Journal of the American Medical Association*. Wide publicity was given to a report of the Council on Drugs of the American Medical Association in which an appeal was made for antimicrobials to be used only when indicated and then in large enough doses to combat the offending secondary organism. Relevant material was sent to state medical journals. Appropriate articles were published in *Today's Health* and special articles from the president of the Association were given to newspapers. Officers of the American Medical Association and members of the Committee on Influenza participated in television programs.

On the state and community levels there were various degrees of activity but, on the whole, the response was good. State and county medical societies joined with other organizations (health agencies, hospital associations, civil defense groups) in making plans for the optimal use of facilities and personnel. Through various media the public was informed as to what might be coming, and instructions were given as to what to do. Men, women, and children were urged to get vaccinated, and an effort was made to have the vaccine available for those who wanted it. It was only natural that in a wide-scale program of this type shortages of vaccine occurred, but these were reduced to a minimum through the voluntary cooperation of manufacturers of vaccine, public health authorities, and local planning groups.

The person who was in closest contact with the disease and the one who played a dominant role in the program was the practicing physician. It was he who gave the assurance on the local level as to the value of the vaccine and encouraged its use. It was he who saw the patient and made the diagnosis. It was he who prescribed the treatment. It was he who watched for and dealt with the complications. It was he who allayed the fears of the public and helped to ward off any suggestions of panic. How much his efforts affected the course of the epidemic is hard to tell. Was the low incidence of complications and the low mortality rate due primarily to a relatively weak strain of virus, to the giving of the vaccine, to the use of antimicrobials, or to the service rendered by the attending physician? I do

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not know, although I am sure that each of these factors played a part. I am also sure that the American public felt much safer because the physician was on the job.

From the account given, it can be seen that the Asian influenza program was actually an emergency medical service program, a type of civil defense, carried on through existing agencies and organizations with full cooperation between governmental and civilian personnel. It was a crash program and undoubtedly some mistakes were made, but in the main it was a job well done and from it valuable experience was obtained.

What of the future? What role should be played by medical organizations and by the individual physician? Do we need to repeat the activities of the 1957 campaign each time influenza threatens to invade parts or all of our country? If not, what should we do to minimize the danger and protect the public?

I would like to present certain suggestions for consideration. In so doing I am speaking as an individual and not in any official capacity. (Neither the Board of Trustees of the American Medical Association nor its Committee on Influenza has submitted any recommendations on this problem.)

I suggest that a national plan of action be developed by a joint committee composed of representatives of the Public Health Service, the American Medical Association, the American Hospital Association, and such other national organizations as might be indicated. The plan should be based upon the experience of the 1957 program, but it should be more in the nature of a continuing process than of a crash program.

On the national level, I would ask that provision be made for: (*1*) Educating the public as to the value of influenza vaccine and the need for its being given before the disease appears; (*2*) informing the public of the imminence of an epidemic and giving advice as to what to do when it comes; and (*3*) acquainting the public, in the presence of an epidemic, with what is being done, so as to allay fears and panic.

For those working on the state and community levels, I would urge that they consider seriously: (*1*) The importance of cooperation between health agencies, civil defense groups, hospital associations, and medical societies; (*2*) the need for a continuing appraisal of existing and potential medical facilities and personnel with a stand-by plan for rapid utilization of such facilities and mobilization of personnel, should the need arise; and (*3*) the desirability of establishing good channels of communication so that information relative to influenza may be channeled promptly to the public.

I would request that special effort be made to supply those who work in the health field—particularly the practicing physician—with the latest information relative to the incidence, the clinical and pathologic picture, the diagnosis, the treatment, and the prevention of influenza.

What type of vaccine or vaccines are available and when should they be given? Should vaccine be given annually? Does an attack of the disease establish an immunity and, if so, for how long? What laboratories are available for establishing the specific type of influenza and what materials should be sent to them for study? Is it essential that the type of influenza prevalent in a community be determined? Under what circumstances should antimicrobials be used and which are the ones of choice? Should the giving of influenza vaccine be included as one of the routine immunizations of infants and children? What is the stature of live virus vaccine? These are some of the questions which confront the physician today as he deals with his patients.

The answers, if they exist now or as they are developed, should be given through the *Journal of the American Medical Association*, state medical journals, and through special communications from organizations or societies which are immediately concerned (i.e., Academy of General Practice and Academy of Pediatrics). As an example of what I suggest, I would refer to the recent article by Thomas Francis on influenza (M. Clin North America, 1959, *43*, 1309), which is an excellent review of our present knowledge and one which should be available to every physician who deals with the disease.

Finally, I would insist upon cooperation and mutual endeavor. An epidemic of influenza is a threat to the health of our people. This threat can be met best, not by enactment of special legislation or by governmental directives, but through the combined planning and cooperative effort of those who work in the field of health—the virologist, the epidemiologist, the hospital administrator, the nurse, the medical technician, the public health official, and the practicing physician.

THE ROLE OF HOSPITALS

EDWIN L. CROSBY[1]

In 1957, I had the privilege of serving with Dr. Burney as the delegate to the Assembly of the World Health Organization. At that time the technical discussions were about hospitals, and we learned many things about the international aspects of hospitals. However, one fact stands out in my mind, and that is that a third of the population of the world has no hospitals. Thus, as far as the future is concerned, a third of the population has no hospital problem in the event of influenza. I shall not try to prophesy because, as you know, he who prophesies is either a fool or will be proved to be a fool. However, I should like to point out to you, through what has been done—what we know now—what the future might hold insofar as hospitals are concerned.

This morning I am glad to represent hospitals, in the first place as a chief executive officer of the American Hospital Association representing the hospitals of the United States and Canada, and, second, as the first vice president of the International Hospital Federation.

In the past two decades we have seen explosive scientific progress in all spheres of endeavor. Hospitals have felt the full effect of these advances in science, especially in the field of medicine and in management. We have done our best to keep pace and have found it most difficult at times. We have to provide the necessary facilities and the skilled personnel primarily for the care of the sick, but also, and most importantly, for research and education. In this connection, I think it is important for you to recognize that the percentage of space in a hospital which is assigned to direct patient care is constantly decreasing. Look around here in this great clinical center and you will see space required in this hospital for the adjunct facilities—for both research and education.

Collectively, for the American Hospital Association and for the International Hospital Federation, we see our role as one of continuous awareness of all aspects of the problems involved: playing our part in the dissemination of information to the public and the hospital field, helping to provide the necessary facilities, with laboratories and their staffs taking an active part in the epidemiologic investigation and surveillance mechanisms. The individual hospital must maintain facilities for the treatment of cases which require hospitalization for both regular and infectious admissions, at the same time providing the essential community service both within the hospital and in the field.

Under the strategic umbrella which is expected from our Public Health Service and from medicine, the actual tactical operation in the hospital remains a matter for local decision with strong national and international leadership. Such action can only be under the direction of the organized medical staff of the hospital in conference with the health authority and hospital personnel and administration. Although much has been achieved in regional and community coordination, the individual hospital still must move within its own organized medical staff in meeting any situation which presents itself. The weapons available must be used intelligently, depending upon the circumstances and, although general guides can be provided through our network of intelligence and information, decisions must be made locally on situations which may vary from mild sporadic cases to epidemics with high attack rates and high mortality.

There are two things the American Hospital Association has done which I think are important in this connection. The first of these is the development of what we call the "Disaster Planning Committee." We saw a series of natural disasters here in the United States shortly after the close of World War II. As an outstanding example of poor planning, we investigated the flood in Connecticut, and we found such unusual things as the Fire Department not being able to cross a river because the National Guard would not allow it to do so. We were pleased, however, that the local hospital did well in taking care of the sick and injured, for it had a disaster program. And when Julian Price was the chairman of the Joint Commission on Accreditation of Hospitals, that important Commission required that all hospitals should have an active disaster

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committee, which not only would have a program, but also would have an active plan. In this way we believe that, through that type of organization within our hospitals, we can take care of any unusual situation, whether it is natural or unnatural.

Today's discussion, however, revolves around another important committee of the American Hospital Association. It is known as the Infections Committee, and is under the chairmanship of Dr. Dean Clark, General Director of the Massachusetts General Hospital. As Dr. Price indicated to you, we worked closely with the American Medical Association and the Public Health Service. We, as the American Hospital Association, issued two bulletins on Asian influenza: one on August 22, 1957, and another on December 18 of that same year. The information and advice given in these bulletins are still essentially sound, and I should like to outline for you the main points which we made to hospitals.

First of all, as Dr. Price indicated, we gave generalized information with regard to the disease, its nature and progress, its symptoms, and suggested generalized principles of treatment.

Second, the use of a vaccine was recommended and, where indicated by short supply or emergency conditions, selective administration was suggested for essential staff and essential personnel of vital community services as well as the aged, the young, pregnant women, and those suffering from debilitating disease and those with pulmonary and cardiovascular conditions.

Third, we recommended special medical staff committees in order to incorporate all other pertinent disciplines and department heads—these committees to direct the over-all approach in coordination with the community public health authorities, the medical society, and the other hospitals of the community. We recommended that the local health officer be a member of this committee of the hospital staff. We stressed the potential available through voluntary services. This is a fundamental approach to obtain the necessary services in the event of an epidemic. We recommended emergency revision of admission policies, it being generally accepted that under epidemic conditions, uncomplicated cases of influenza will *not* be admitted, and that elective work also will not be admitted; only emergency work will be admitted. We emphasized the need for extensive isolation practices and the review of the situation as it presently existed in the hospital. We recommended review of treatment procedures, especially the use of antimicrobials and, of course, in hospitals, the need to maintain an adequate supply of supportive equipment and drugs. These were the recommendations which we made then and which we still believe are important insofar as the control of epidemics is concerned in the hospital field.

There is one recommendation we made which is debatable. It was whether or not visitors should be admitted to the hospital. We recommended that they should not be. There are many people who believe that this is wrong, but I still think that our view was correct.

As I indicated, it is dangerous to attempt to predict the future, but there are certain trends which I am sure will continue. I feel confident in predicting the following: First and by far the most important, I see a closer brotherhood of nations, each sharing its epidemiologic intelligence and other scientific knowledge to a degree which has never before been known. Second, I see a far greater integration of the various disciplines in the health field, all working in closer harmony at all levels of organization. I see a health field much better informed than it is today, working with better tools to serve the public, which itself is better informed.

Without doubt, research and development in the scientific fields will produce many other new things. Regardless of what else it produces, the possibilities of the by-products involved stagger the imagination. We can only speculate, but here is our future, and I urge all to foster in any possible way continued diligent research. I see a more vital participation in surveillance and identification of the disease and in closer relationships with the health profession, a continuing elasticity to meet the changing problems which are to be faced. This includes the development of a greater awareness of community responsibility for hospitals.

I hope you realize that the voluntary hospital field of this country is made up of only 120 hospitals which have more than 500 beds. Two-thirds of the hospitals of this country have less than 100 beds, and this creates a great problem for all of us who are concerned. Finally, I see a general regionalization of hospitals in the United States. This was recommended in the Hill-Burton Act in 1946. We have not seen much advance, but we need regionalization of hospitals by community, by area, by state, and by groups of states in which the essential facilities required for research, diagnosis, and treatment will be so grouped to provide maximal efficiency in the

over-all problem of disaster planning and epidemic control. I should like to express my pleasure on behalf of the American Hospital Association for being present at this conference, to exchange views and share experiences with so many distinguished people. Whereas one cannot be so naive as to be complacent with the feeling that problems of epidemic influenza are solved, I think that we can be assured that we are gradually getting nearer to our goal when the hospitals pledge their support in any way possible in attaining this goal.

THE IMPACT OF INFLUENZA ON INDUSTRY IN GENERAL

W. P. SHEPARD[1]

Absence and Automation

Any disease which causes disability even of short duration in sudden epidemic form is of increasing concern to industry. Influenza in particular, with its high incidence, short incubation period, and sometimes high virulence, is a dangerous combination for industry—more so today than ever before. Mechanization (including electronics) together with belt-line production methods mean that absenteeism due to illness can quickly reach a critical point at which the industry is badly crippled. This critical point varies in different industries and we shall consider specific industries later.

Importance of Key Personnel

Generally speaking, an absence rate of 20 per cent even for a short time is serious for most work places. This is especially true among personnel in key positions. The effect of mechanization is that fewer and fewer people are responsible for more and more output. In one industry, 120 people operate three electronic machines which will eventually be doing the work of 3,000 clerks. Simultaneous absence of twenty-four of these people for an average of three days would seriously cripple this industry employing nearly 70,000 people. In most cities, police and fire departments are seriously hampered with a 20 per cent absence rate. Among hospital personnel, a 20 per cent absence rate is serious, especially since the admissions rate is usually excessive at the same time.

The Job of the Medical Director

The medical director in an industry must, first of all, have an accurate system of keeping a record of absences of his company. Next, he must, with the help of the personnel division, identify those groups in key positions who are especially important to the industry's output. Third, he must decide and sometimes convince his management that the particular industry is or is not essential to the nation's economy and, if not, that they could suffer a reduced output for two or three weeks without serious effects. This is not always easy to do, but it is especially important when supplies of vaccine may be limited.

Next the medical director needs, and in recent years has received with remarkable accuracy, the following information:

(*1*) The strain of the virus involved, the attack rate, and whether it is increasing or decreasing as the epidemic sweeps the country
(*2*) Some idea as to the virulence of the strain
(*3*) The effectiveness of vaccine and how soon it becomes effective
(*4*) The percentage of expected disabling reactions.

The experienced medical director realizes that absence rate due to reactions varies considerably from industry to industry. Where morale is low, those temporarily disabled with reactions may be twice as many as in an organization where morale is high. Nevertheless, it is a fact that in some industries, reactions may actually add to the absence rate, especially when the immunization program is started late.

Whether to offer vaccine at company expense is a decision which should rest solely upon the recommendation of the medical director. In 1957 this decision was sometimes taken by the chief executives of the company without consulting the medical director. This will not happen if the medical director has the authentic information mentioned above and submits early and periodic reports and recommendations to his chief executives.

It is also the medical director's responsibility to consider the effect of immunization at company expense upon the medical relations of the company as a "citizen" of the community. There are county medical societies which still condemn any such procedure under any circumstances. When this happens, it makes it difficult for the medical director to continue his good relationships with the practicing physicians in the community, relationships which are so important in the treatment and rehabilitation of his disabled employees.

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American Medical Association Policy

It is hoped that such county medical societies will be a little less adamant as a result of the approval of the Board of Trustees of the American Medical Association last December of a statement on immunization in industry (1). This points out that health maintenance is primarily the responsibility of the individual person. The employer, however, has an obligation to provide a safe work environment for his employees and he has a valid interest in the prevention of loss of time and efficiency resulting from his employees' ill health. He may, therefore, feel justified in providing certain preventive health measures in a given situation where the employee, the employer, and the community stand to benefit. It is suggested that an employer should provide immunization procedures to employees who, by reason of their occupation or job assignment, may be exposed to significant hazards against which immunization procedures are available.

An employer may provide immunization procedures in the face of an impending epidemic which threatens to interfere materially with operations and against which immunization procedures could not be applied by community health resources in time to provide optimal protection.

It is suggested that county medical societies under these conditions should be informed of the situation in a given industry, and to the extent feasible and practicable local physicians should be given an opportunity to participate in the industrial immunization program.

In addition, an employer may wish to participate in community-wide immunization programs at the request of, and in cooperation with, a local medical society or other community health agency.

Industrial Immunization in 1957

There are a very few authentic reports to help evaluate the effectiveness of immunization with the monovalent Asian influenza vaccine in 1957. It was in short supply, probably not as potent as it should have been, and was given in a variety of dosages and methods. These varied from 0.1 ml. intradermally to 1.0 ml. subcutaneously.

My own company did not offer immunization at company expense among its 17,000 employees at the home office. Another life insurance company with a smaller number of employees but much the same type of operation did immunize a large percentage of its employees. As nearly as we can make out, there was no difference in the absence rate between the two companies. Our absence rate reached a peak of nearly 15 per cent for the total group but, strangely enough, different divisions, not contiguous, "peaked" at different times, varying as much as six weeks. There was central, sealed air-conditioning in the two main buildings, but none in a third smaller building. There are centrally operated cafeterias for luncheon, but the members of each section go to lunch together at a regularly designated time each day.

Effects of Influenza on Life Insurance Companies

As to the specific effects of influenza epidemics in the life insurance business, they are mainly reflected in increased claims. The last four months of 1957 produced a mortality rate from influenza and pneumonia among our policyholders four times that of the like period of 1956 (2). The increased mortality was mainly among the elderly, the very young, and those with chronic illness. Death rates from cardiovascular-renal diseases were 17 per cent higher in the fourth quarter of 1957 than in the like period of 1956. For the entire period from October, 1957, to April, 1958, the total death rate was 12 per cent more than the death rate of the year before. For the cardiovascular-renal diseases alone, the rise was 10 per cent.

In the 1918 epidemic, the Metropolitan Life Insurance Company received about six times the usual number of death claims each day for several weeks. We paid 68,000 death claims from influenza and pneumonia amounting to $24,000,000 above what was normal for the period. Every available clerk was transferred to the claim division, which worked around the clock.

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ESSENTIAL INDUSTRY AND ITS ROLE IN AN INFLUENZA EPIDEMIC

NORMAN PLUMMER[1]

It is a genuine privilege for me to be on the program at this Conference. This is the first time in a number of years that I have joined many of my former colleagues with whom I was associated in carrying out research on influenza and other infectious diseases. This is a pleasure and indeed it is a privilege to join this distinguished panel to discuss the important subject of the role of health organizations in future influenza epidemics.

The chief role of an essential industry in an influenza epidemic is to continue to furnish to the community those essential services whose unavailability would endanger the health and welfare of that community. This means that the employees of that industry must remain healthy and productive in order to accomplish the primary objective.

In the United States, of some sixty million people in non-agricultural employment, there are approximately twenty million who are employed in essential occupations, namely, those having to do with protection (policemen and firemen), health (doctors, nurses, hospital workers, et cetera), transportation, communications and other utilities, and food and fuel processing. Today, the essential industries for the most part have well-organized preventive medical services and are equipped to carry out many of the procedures that we know are necessary for the control of an influenza epidemic.

I am not prepared to speak specifically for all industry, but I can assure you that I am convinced that in an emergency, the social conscience of American industry would produce Herculean efforts in the public's behalf. I can give you an idea of what would be done in the company with which I am associated, The New York Telephone Company. As the name implies, we operate in New York State and, along with eighty-eight smaller, independent telephone companies, provide the state's service. We employ about 75,000 people. We have approximately 55,000 employees in New York City, and Nassau and Westchester Counties. We have another 4,000 employees in Buffalo, 4,000 in Albany, 2,500 in Syracuse, and the remaining, approximately 10,000, scattered through most of the smaller cities in upstate New York. What would be our program in case of a serious epidemic?

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Employee Information

The employee information program of a large industry is almost as extensive and complex as are communications in the service. In the New York Telephone Company, we publish a company magazine monthly and three territorial newspapers every two weeks. In case of an epidemic, these publications, along with management bulletins, would carry to all of our employees general information on preventive measures, immunization, and early treatment. Top management's understanding of the situation would be expressed to all employees. The essentiality of telephone service would be emphasized. Communication with our union leaders would be important.

Memoranda to the supervisors and a letter to each employee would outline the details of an immunization or other preventive program to be carried out. In all organizations, good two-way communications by word of mouth between individual employees and their supervisors become vital for a good understanding of a situation facing the company.

Influenza Surveillance

Supervisors have a set of instructions for reporting through lines of organization on absences. This is usually done on a weekly basis and these figures are compiled into a detailed report of absence on a monthly basis. However, if it becomes important, absence can be reported into headquarters on a daily basis and compiled on a weekly or even a daily basis. The Bell System furnished such a weekly compilation in the thirty-six largest cities in the United States for the United States Public Health Service during the 1957 epidemic. I have been informed that this proved to be highly useful in nation-wide surveillance of illness at that time. At all times our supervisors are instructed to report unusual

health situations within offices to our headquarters group. This means that, through these different reporting methods, the beginning and progress of an epidemic can be recognized.

In the 1957 epidemic our reporting showed scattered small outbreaks as early as September 15. There were increasing absence rates to about October 15, and then a rather rapid drop with a return to almost normal attendance conditions by November 1. Our reporting methods also picked up the increased incidence of serious illness throughout the winter and spring of 1957 and 1958.

In the company's influenza surveillance program, in addition to the information received from our absence and illness reports, we are able to make a more medical type of observation and analysis in our three medical dispensaries located in Manhattan, Brooklyn, and Albany. In these locations our doctors and nurses, at the time of an epidemic, will see between 400 and 500 employee-patients each day. In these medical departments we keep a record of all febrile cases, and an increasing incidence of these has meant in each instance the beginning of an influenza epidemic. On our dispensary cases, at times, in cooperation with the New York City and New York State departments of health, we have carried out virologic and serologic studies for the identification of specific infection. Additional clinical studies, such as blood counts and chest roentgenograms, can be made on the employees when indicated.

Immunization

In 1957, as rapidly as vaccine could be procured, on a voluntary basis, we offered immunization to all of our employees. We gave about 54,000 injections to approximately 65 per cent of our employees. This was done by teams of doctors and nurses going directly into our various work locations. In an organization such as ours, the scheduling and administering of vaccine can be carried out very efficiently, with employees away from their posts for a relatively few minutes. In 1957, and in other experiences with influenza vaccine, we had almost no problem of reactions.

All of you remember the vaccine shortages of 1957. In our company this had a decided bearing on the percentages of employees volunteering for immunization. We started vaccinating on September 23 and continued as rapidly as vaccine became available to us. At the start of our program more than 90 per cent of the employees in the groups included volunteered and received vaccine. In the final weeks of the program, when the epidemic was subsiding rapidly, the percentage dropped off to about 25 per cent.

From our experiences, providing that vaccine and professional personnel were available for our program, I believe that our employees in major concentrations could be vaccinated within one week and that the program throughout the company could be completed within two weeks. Furthermore, with serious threats of an epidemic, I believe that more than 90 per cent of our employees would volunteer for immunization.

Treatment and Hygiene

Although the company health program is one of preventive rather than therapeutic medicine, through our extensive employee information program and Medical Department services, much helpful advice and counsel is given to our employees regarding general hygiene and other preventive measures as well as general advice when illness occurs. This service can have particular value at the time of an epidemic when the practitioner and the hospitals are fully occupied attending to those seriously stricken. Furthermore, if in a future epidemic, should Streptococcus and pneumococcus and other secondary bacterial invaders get on the loose as in 1918, I can see where industrial medical facilities could be most effective in carrying out a program with the use of the sulfonamides or other antimicrobials.

Analysis of Results

In the 1957 epidemic in the New York Telephone Company, our personnel record methods made it possible to evaluate the epidemic in terms of numbers and distribution of cases as well as to some extent to evaluate the usefulness of vaccine.

At the time of the epidemic, the company statisticians arranged for the analysis of the absence records on 4,409 employees selected at random from the entire group of 75,000 employees. The period of this study was from September 23 to December 15. We found that 2,331 of the group were vaccinated and 2,078 were nonvaccinated. Approximately 35 per cent of the vaccinated group had respiratory-illness absence

for an average of 2.7 days, whereas approximately 41 per cent of the nonvaccinated had respiratory-illness absence for an average of 3.3 days. While the difference between the two groups is significant statistically, we must appreciate that the groups are not the same as alternate control groups and that they do have a degree of bias which cannot be accurately ascertained.

The statisticians also were interested in the financial savings of such a program. Using the above figures on decreased numbers of cases and length of illness applied to our entire company population, assuming a 70 per cent vaccination rate, the saving would be approximately $185,000 in wages and salaries. With a 100 per cent participation rate, the savings would be approximately one-quarter of a million dollars. The out-of-pocket cost for carrying out the vaccination program was of the order of $50,000.

During the 1957 epidemic, the shortages of vaccine made it possible for us to appraise the effect of vaccination in another way. It happened that in eight of our Manhattan-Bronx-Westchester accounting offices, representing 1,629 employees, the vaccination was given before October 2. On the other and, in another eleven comparable accounting offices in the same territory, with 1,783 employees, vaccine was not given until after October 18, when the epidemic was subsiding rapidly. The absence rate during the month of October of employees in the group of offices in which the vaccine was given early was 6.35 per cent compared with 9.68 per cent in the offices given vaccine later. This is a difference of 52 per cent and it appears to be a highly significant figure.

The medical literature has reports on influenza vaccination during the 1957 epidemic from a number of industrial concerns. Dr. L. Holland Whitney (1) reports that 416,000 Bell System employees from coast to coast received vaccine and that the evidence of significant reactions was negligible. Comparisons of results in the vaccinated and nonvaccinated groups in a number of Bell System companies, as well as in a number of other industries (1–5), have been reported and were uniformly favorable. However, none of the studies was strictly controlled in the manner carried out in many instances in military service and institutional groups.

Because of the discussion in this conference of mortality following influenza, I should like to refer to a study by Benoit and Goldstein (6) carried out in the approximately 5,000 employees of *The New York Times*. Almost 50 per cent of the group requested vaccine and received it before the epidemic started. The other 50 per cent received no vaccine. In the entire group, they recorded twenty-one complications, including ten cases of pneumonia, six of tracheobronchitis, four of severe asthenia, and one of otitis media. Twenty of these complications were in a nonvaccinated group and only one was among those vaccinated. Perhaps vaccine is more effective than we suspect in preventing influenzal complications and deaths. The discussion at this conference makes it imperative that this phase of the influenza program be studied more carefully. I believe that some of these answers can be found in studies in industrial groups, particularly because many of our companies are introducing machine methods for the tabulation of personnel records, and such statistical analyses as these can be readily made.

In concluding my remarks, let me reiterate that the Telephone Company's primary objective is to maintain effective and continuous communications service. An epidemic that felled a great majority of the population would, of course, complicate the task. Some of our confidence in our ability to continue to give service rests in the high percentage of dial telephones we have today. This equipment would care for all the calls that people would dial direct, and that covers quite a few places these days. We are confident that our equipment would operate satisfactorily for quite a while with a reduced maintenance force. A combination of mechanization, morale, and medicine would, we hope, help us to weather the storm.

In carrying out our objectives during an influenza epidemic, it is apparent that our own employees would benefit by our health maintenance program. Also, the community, in addition to retaining communication service, would gain by the immunization of a large group of people, and by health information and education disseminated by employees into their homes as well as widely into the community.

Finally, on behalf of one essential industry, may I offer the assurance that, if influenza causes homes and hospitals to become isolation centers, that isolation will not be complete and the telephone will still be a valuable servant.

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THE ROLE OF COMMUNICATIONS

Television, Radio, and Others

ROBERT S. KLECKNER[1]

Dr. Dingle, I would like to pick up where my fellow-displaced Hoosier, Dr. Burney, left off, and comment on a couple of other problems of communication mentioned by others during this meeting. Dr. Burney observed that the failure to develop effective local information was an "Achilles heel" in your programs. Previously, Dr. Korns observed that most frequently he learned of influenza outbreaks through the newspapers, and one of your other young men, Dr. Rosenstock, said that the failure of many communities to put on effective influenza control programs was because the medical society officers sometimes used a "pocket veto."

I have been both pleased and shocked at some of the things that I have heard during this session. I have been pleased with the reports of the research. I have been pleased with reports of epidemiology. I am pleased even if these reports do not filter down in many instances to the local physician who sees the patient and sets the policy. However, I have been shocked quite a bit at organized medicine, of which many of you are a formal part. Some of you are also in touchy situations as far as effectual participation in your local medical societies is concerned.

I am supposed to discuss communications, particularly newspapers, radio, television, et cetera, all of which are media—usually entirely voluntary and free—used in health emergencies. I am not certain that, in many health matters, the mass media are not in the same unfortunate position that Dr. Payne of the World Health Organization mentioned, i.e., everyone being two months late in identifying the Asian strain because the Chinese did not tell anyone.

Let me outline the problems of newspapers and other media, and then let us see if there are suggestions for solution of them which will be of mutual advantage. I live and work in Chicago. On February 1 or 2, I called the Chicago Board of Health to try to find out how much influenza we had had during the month of January, and naturally I had to get clearance from Dr. Herman N. Bundesen, the late head of the department, before I could get to the biostatistician, Dr. Frank Bauer. I asked him how many cases of influenza there had been reported for the previous month. For the whole city of Chicago, he said that seven cases had been reported and three deaths from influenza. This was for the whole city of approximately four million. Then in checking back through pneumonia records, I discovered that in August, 1959, there were 150 pneumonia deaths, with only 141 cases reported to the Board of Health. True, some of these infections may have been in patients reported as cases a month before. However, this type of reporting not only makes medicine look silly, but it throws off the whole national picture because the statistics that Dr. Carl Dauer has to disseminate are based on reports which go to the state, and then to the national office. I happen to know from personal experience that Dr. Anderson has had to make telephone calls to supplement the statistics that the states report because he was not quite sure what the statistics meant.

I assume that the health statistics from Chicago are no worse or no better than anywhere else in the nation. Now, from a communications standpoint, this means that the news media themselves are possibly being more alert to public responsibility and have had the job thrust upon them of trying to find out when there is a health emergency—say a sudden upsurge of "flu." How do we know that there may be an emergency? Well, first of all, the nurse at the *Sun-Times* calls me and says, "Bob, there is greater absenteeism this week than we had last week. Do you think there might be something in the wind?" So then we assign a couple of reporters to make on-spot checks on absenteeism in schools, business, and industry. This takes a couple of hours to get a fair sample, but we do it, and then call the health and school authorities for comment. This is a kind of reverse procedure, I would suggest, but a routine one.

In Chicago, for example, the Board of Education gets in its main office only monthly reports on absenteeism. This means that the newspapers

[1] Science writer, *Chicago Sun-Times*, Chicago, Illinois.

have to call the individual schools and get the superintendents to find out about absenteeism at any given week. So you see, just like some health departments, some school departments do not know what is going on health-wise. Now because of newspaper pressure this system has been revamped and as of last week they were starting to get weekly reports to the central offices of the Board of Education. We admit that collection of information is a job for mass media but, on the other hand, I submit that we go beyond the call of duty in instances like these, when, instead, health information should be telephoned regularly and accurately by duly constituted health agencies.

Newspapers, radio, and television are essential weapons in education for public health and in combating possible epidemics. And they all stand ready to cooperate, as was certainly illustrated in 1955–1956 in the all-out war against polio, and to assist in needling the public—and some doctors—into a mass Salk program. As you know, we have helped and will continue to help in every way we possibly can in emergency situations, but you are going to have to give us the ammunition first before we can fire it.

What can be done currently? First, I would say recognize that communication begins at home. Next, I would suggest an all-out educational campaign within the local medical societies as to the need for this immediate reporting of influenza and influenza-like maladies to local health officers who, in turn, make it available to the state, nation, and news media. In this way, perhaps, trends will be recognized earlier. Thus warnings can be given. Preventive measures can be taken. Lives may be saved. I do not think many doctors realize that reporting illnesses is a part of saving lives. Just how this can be accomplished, I don't know. Perhaps sickness forms which doctors do not fill out should be simplified so that they will have less excuse not to do so. If you can lick this problem, you have started a long way along the lines most of you have been thinking, but have not yet attacked successfully.

In Chicago, as some of you here know, there have been communication difficulties between the mass media and hospitals. Most of the hospitals like us only at the time of their fund drive. If there were a bad accident in which many people were involved, reporters, photographers, and radio or TV had a most difficult time. If you were assigned to check an outbreak of food poisoning within the hospital itself—well, you can imagine what happened!

We finally set up a cooperative committee between hospitals and the news media. I was on that committee and, as I recall, it took more than two years before we worked out a code that was mutually agreeable. But it is working. As you can see, your headaches also are our headaches. I think we can help each other, if we sit down and try to work these things out. I know that the great American way of living is a committee, but maybe we can get an effective type of communication between us, so we will get communication from the bottom up. As Rosenstock emphasized, this is all on the local level, and a lot can be done with the blessing and cooperation of the local medical society. I think a continuous, well-planned missionary job there will be a step in the right direction. The national policy on an epidemic may be outlined and suggested, but there is no follow-through from the doctor who advises his patient and says, "Well why worry, it is only influenza." I don't think it is an organizational job for the newspapers, radio, or television alone; but I can assure you all, we will be happy to report on its solution, which will come when you and we learn to work together.

General Discussion

Dr. John H. Dingle (Cleveland, Ohio): We have now come to the time for general discussion. I am delighted that we have with us this morning one who was quite instrumental in instigating and formulating the program for this conference. We were all distressed when we learned two days ago that he was having his own personal experience with influenza, but this morning he is here. He says he thinks he is going to live and is not too unhappy about it. I would like to ask Dr. Smadel to open the discussion. I would like further to exert the prerogative of the chair and ask him to comment on any part of the program that he wishes, because I know he will do it anyway.

Dr. Joseph E. Smadel (Bethesda, Maryland): Dr. Dingle, you know I am really more than a little bit embarrassed about having been absent from the Conference on Wednesday, when I was flat on my back. I think it must have been "flu," but I will tell you later.

The advantage of having all of the health organizations present their respective responsibilities as a group is most important. It is not as unique, in the opinion of the planning group, as the opportunity to bring together many outstanding individ-

uals who have responsibilities in health organizations of various kinds in this country, and provide them with a forum to express what they know and what they intend to do. It seems to me that a number of things have been very clearly brought to our attention today. I hope we have learned from the things that have been told us.

At least, if we have not learned, we will have recorded the information and advice so that those who follow may learn. Perhaps the really important thing in a meeting such as this is that we have, I hope, increased the small group of dedicated virologists and immunologists who will be willing to worry about pandemic influenza during the long dry period from one pandemic to another. The mere fact that we have been able to bring people from hospitals, industry, the state and territorial health groups, and the communications group, together for discussion of mutual problems today, means that we have added to this nucleus. I only hope, Dr. Dingle, that we do not lose our interest, that we are able to maintain it, so that they and their successors will be available when the next pandemic occurs.

Dr. N. C. Kiefer (New York City, New York): I would like to elaborate a little on some of the statements in Dr. Shepard's paper, which I read. The American Medical Association's Council on Industrial Health has a committee on industrial health emergencies, of which I am the chairman. In 1958 we sent out questionnaires to sixty-nine industries scattered around the United States. Now this was done chiefly to evaluate professional administrative problems associated with vaccination and, as a matter of fact, it led to the American Medical Association's statement of policy on immunization in industry that was mentioned in Dr. Shepard's paper. However, I think you may be interested in some of the results. Sixty-five companies responded and fifty-two of these took an active part in providing and/or administering vaccine, all on a voluntary basis, to a total of 620,000 employees or about 43 per cent of all of them. Seventy-five per cent received the vaccine subcutaneously, 24.5 per cent intradermally, and the remaining 0.5 per cent by a combination of the two or some other means.

Absenteeism rates were higher than those for the comparable period in all but one industry, and during the peak period ranged to as high as 150 per cent increase over a comparable period of the preceding year.

Although evaluation of the effectiveness of the vaccine was not a primary objective of the study, twenty-seven medical directors did respond to a question on this subject. Twenty-one of the twenty-seven stated their belief that vaccination had reduced the incidence of influenza, and some of these beliefs, at least, apparently had been based on a reasonable statistical evaluation. One, for instance, reported four times as much influenza among the nonvaccinated. Another reported a 65 per cent reduction in absenteeism among the vaccinated. Another reported the ratio of absence of those vaccinated to those nonvaccinated to be 4.2 to 1. And another reported a 40 per cent reduction in respiratory illnesses in the vaccinated group. Similar to Dr. Plummer's observations, there was a general impression that the vaccine not only had reduced the number of cases but also had reduced the severity and duration of the disease when it did occur among the vaccinated.

There is one other point that I would like to mention. I am somewhat concerned over the recent experience we had in the Equitable Life Assurance Company just last month. We offered vaccination only to a small group of key employees, including, as you might expect from Dr. Shepard's comment, the people associated with our electronic computers. It was startling to learn that 93 per cent of the 261 employees who participated developed sore arms, while 47 per cent of them reported mild to moderate systemic symptoms, and another 65 per cent reported these symptoms as severe. Furthermore, eight employees (3 per cent) missed some time from work. We did not have more than this number in 1957 when we vaccinated over 3,000 people. In 1957 we used a vaccine of Asian strain only. This year we used polyvalent vaccine. Otherwise, the methods and techniques were identical. Although this total time loss from work is admittedly a small price to pay for the anticipated protection, at the same time the adverse psychological effect was demonstrated by the fact that we offered booster shots two to four weeks after the original vaccination and only 50 per cent of the employees returned.

Dr. Alfred Evans (Madison, Wisconsin): I would like to make one or two comments. The first one is in relation to my responsibility as a director of the State Laboratory. I think laboratories such as ours have the opportunity, if not the responsibility, to carry out programs of what one might term "serologic surveillance." This might take one or two forms. It might be either the regular testing of serum submitted for respiratory disease diagnosis, such as Asian or other influenza strains, or it might take the possibility of testing randomly selected pairs of serum sent in for serologic diagnosis. Such activity, I think, is one that should deserve sponsorship, because it may be difficult for such a survey to be carried out without assistance. Indeed, I think that Dr. Andrewes' suggestion to establish an international network of laboratories is an excellent idea. It is one to which some financial assistance would be very useful so that this type of routine surveillance could be carried out not only

for influenza but for other key epidemic infections. Through some such mechanism of serologic surveillance, one might be able not only to determine the early occurrence of epidemic disease, but even to get some guide lines as to the needs for vaccination and knowledge of the rise and fall of different influenza strains.

The second comment I have to make is while "wearing the hat" of a teacher of preventive medicine. If you had a head like mine, you can see why two hats might be necessary to keep warm. In this framework, I am impressed with the disparity of the suggestions regarding vaccination. On the one hand, there is that dealing with vaccination from "womb to tomb" every year in order to build up a broad antigenic base and, on the other hand, that of waiting until an epidemic occurs and trying to provide vaccine prepared with the new strain within the shortest time possible. It would seem to me that there should be some operating ground between these two discrepant positions. Such a compromise might be the routine immunization of the essential workers, infants, the aged, pregnant women, and those with respiratory disability. The routine immunization of such persons before periods of high prevalence of influenza such as we have now would be important and effective. We certainly have evidence, I think, that there is a pool of susceptible persons who were not attacked by influenza in 1957 or 1958 or, at least, in whom the antibody had dropped to such a low titer as to be ineffective in preventing infection. It would seem to me that some program of routine immunizations during periods of high prevalence of these critical workers would at least form an operating basis from which one might be able to proceed when an epidemic strikes. At least this would prevent disability and meet emergency situations if such programs were already organized.

Finally, I might comment parenthetically that Dr. Hirst's suggestion of rapid identification with use of monkey kidney cells for virus isolation has been tried in our laboratory, but I think that the identification by this method occurs at about the same time that the hemadsorption phenomenon is demonstrable, so I am not sure that a great deal has been gained in terms of time.

Dr. A. D. Langmuir: As one who was fairly active in the deliberations during 1957 and as one who does not follow Dr. Crosby's admonitions of not only being a fool to start with but of being proved a fool by predictions which often have been made and often disproved, I certainly was one of the more active people talking against a second wave on epidemiologic and immunologic bases. In the deliberations of December, 1957, and early January, 1958, I was a most vocal person against the recommendations to embark upon a large-scale immunization program, particularly for older persons and for those with chronic respiratory and cardiovascular diseases. On the grounds that this would be basically a program of propaganda, urging people to go to their private physicians, the number would be in the range of maybe 10 or 20 million. The fee they would be encouraged to pay would be five or ten dollars. This would result in a fifty to one hundred million dollar program financed by the citizens themselves for a very—what seemed to me then—doubtful purpose. I also very actively said, if there is a second wave, it will be influenza B and not influenza A, and the available vaccine is monovalent, which was then in adequate supply.

On hindsight, it seems quite apparent that the second wave was influenza, Asian influenza. Although the evidence is yet to be produced to show that vaccination will prevent death, I think, as we have to make decisions in this world, this is a reasonably well-founded one. It would have been wise to have embarked upon a program that might have been as expensive as I outlined in view of the some 40,000 deaths that did occur in that second wave.

Dr. C. H. Stuart-Harris: I confess that I feel quite washed-up this morning, and I feel that this is a kind of domestic program that you have. Those of us from abroad are very interested to see what conclusions your public health officers can come to. I was primarily interested in Dr. Merrill's comments. We do have a tremendous problem in the field of public health and, for once, I am extremely glad that I am not a health officer or anybody who has health attached to his label. I do not really feel that I can give you any help in settling this issue facing you.

Dr. Thomas Francis, Jr.: I would like to comment on the problem which has been discussed so widely and repeatedly since yesterday afternoon. This was actually clarified to my satisfaction this morning both by Dr. Burney's remarks and those of Dr. Smadel, as to what we are going to do about learning of influenza and doing something to improve the situation during inter-pandemic periods. We have, according to the current definition of pandemic, very long periods with lots of epidemics and smaller pandemics. Therefore, I think it is very important to keep alive, alert, and active in working toward prevention.

I am a little sorry that Dr. Andrewes left because this inhibits me a little, just as acting as chairman yesterday afternoon inhibited me a little. However, I did talk with him so this is not something said entirely in his absence. Dr. Andrewes said two things which, I think, really require a good deal of thought. The first is: "One should look at the world as it is." I think this means that it depends upon whose knothole you are looking through as to what world you

see, and that our point of view and our view of public health and preventive medicine should not be governed or directed by a British view of preventive medicine, which is so different from ours. The statement is made commonly, "Well, of course we couldn't do these things in our country." However, we can do them in this country and we are really interested in preventive medicine. I personally must confess that I am interested in preventive medicine and allaying these emergencies rather than serving a period as a periodic fire horse.

The second point that Dr. Andrewes made was that we should not get ourselves confused with a deluge of obsolete antigens. However, I think we should not let ourselves be completely smothered by a deluge of out-dated preventive medicine ideas either and, in this sense, I would like to point out that we are talking two different languages. Smallpox vaccination in this country and in England are entirely different problems. Diphtheria vaccination, Dr. Stuart-Harris tells me, is being largely stopped in Great Britain at the present time because of objections. We do not believe that this would be a sound preventive medical procedure, and it still seems to me that, if we are going to improve the situation of respiratory disease, we have to be just as vigorous about trying to develop our knowledge, our procedures, and our patterns of doing these things as, seventy-five years ago, people had to be in beginning to get clear of the enteric scourges of life. There has to be a new program and a new way of life rather than looking upon this with fatalism and simply sitting and waiting for a pandemic to knock you off your feet.

Dr. C. A. Brandly: I might make a comment or two from the standpoint of those of us who get up each morning worrying about what impact influenza or other diseases which are prevalent may have on the animal population; what effect they may have on our animals in addition, of course, to their direct or eventual influence on the arch-parasite, man. You may recall the report by Dr. J. A. Myers (Dis Chest, 1958, *34*, 650) in which he pointed out that recognizing tuberculosis in animals, particularly cattle, is a case-finding procedure for locating the disease in human reservoir contacts. Employing a stamping out program, the incidence of tuberculosis in cattle has been reduced over the past thirty years so that the tuberculin reactivity is 1.56 per cent as compared with about a 30 per cent prevalence in the human population.

With respect to influenza, as has been pointed out, there is present concern about the reservoirs in the animal population. The over-all epidemiology—or if you wish, the broader term, epizootiology—does have to take into consideration these reservoirs and the ecology of the virus itself. Nature is inevitably concerned about perpetuating the virus parasite as well as the host, and the former may need to pursue devious routes toward perpetuation. It perhaps will bear re-emphasis that viral ecology does not recognize the arbitrary line we have attempted to set up between ourselves and the rest of the animal kingdom. I think we are all gratified by a progressively more realistic appreciation in this direction, since it redounds to the welfare of man. It stresses consideration of the fact that the virus will, if possible and necessary, embrace any and all members of the animal kingdom in its effort to perpetuate itself.

One more comment in view of prior allusions—we tend to be careless in our use of the words "vaccination" and "immunization." They are often employed synonymously and imply that if one is vaccinated, one is automatically immunized. This fallacy has again been refuted by various speakers here, together with the fact a vaccination program alone seldom accomplishes effective control of a disease. With influenza it is a temporary and ephemeral measure by which we may be able to circumvent and survive onslaughts of epidemics.

Dr. Dingle: This ends this Conference on Asian Influenza. I would like to thank all the participants and discussants and express, in behalf of all of us, our appreciation to Dr. Loosli and his committee who planned it.